

Paying the Civil Service Scientist

THE question of scientists' pay is not one for glib editorialising. Within the confines of a thousand words it is simply impossible to give a rational assessment of what should be done to the present simple pay structure for the enormously complex organisation that is the British Civil Service. What one can do is try and see broader issues. The contributions in *Nature* by Dr Leigh (248, 184; 1974) and Drs Gibson and Hawtin (in this issue) deserve careful reading because they clearly set out some of the fundamental questions underlying the present impasse.

In the late 1940s the Priestley Commission established that the pay of civil servants should be removed from the "cockpit of politics". To this end it proposed that parity be maintained with salaries outside the Civil Service and this, despite the inflexibilities of salary scales and increments, worked tolerably well for many years. Scientists were always in a potentially anomalous situation, since although they do not form a large percentage of all civil servants, they do account for more than half of Britain's scientific research manpower when scientists in fringe bodies (whose pay scales are coupled to civil service rates) are included. Far from avoiding pace-setting, which was Priestley's hope, scientific pay rates in the Civil Service cannot but set the pace for all scientists' pay. Up to 1971 the circularity inherent in this system was avoided by establishing broad parities between the scientific categories and their counterparts in the administrative, professional and technical grades. Since then the internal parities have been discarded and external parities sought through so-called 'pay research'. The result of this exercise did not satisfy the Institution of Professional Civil Servants, representing the scientists, and the matter is now with the Pay Board, whose decision is expected within weeks. There is no dispute, however, over the fact that scientists have slipped behind in internal parity during the dispute nor that they have conducted themselves with restraint. It is inconceivable that they should not be well rewarded by the Pay Board or that their forbearance should penalise them.

There are three salient points which need some emphasis: that going off an internal parity has created some nonsensical situations which deserve urgent attention; that the government, in deciding its own scientists' pay, is deciding the status of a profession; and that the role of government in science is worth continued questioning.

Every scientific civil servant can point to anomalies in structure. For instance scientists as they become more senior become more likely to administrate, and to main-

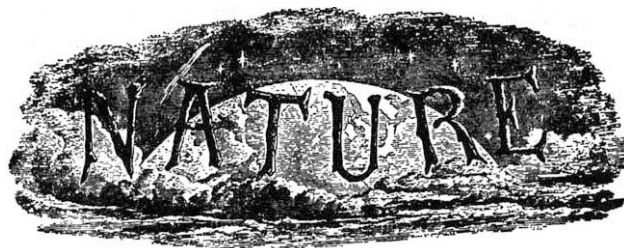
tain a distinction between administrators who have followed different paths to their present position is manifestly absurd. Nor is the anomaly confined to the upper echelons, for many scientists could easily have been classed as technologists and *vice versa*, yet there are now hundreds of pounds between comparable categories.

More important than repairing these frayed edges, though, it is vital that the question of civil servants' pay is seen for much more than that. Like it or not, the Pay Board finds itself evaluating the financial rewards of all scientists and, because of this, affecting recruitment into science as whole and even the decisions that have to be made (as Drs Gibson and Hawtin point out) years before a young person starts work. Thus a decision consciously to differentiate between the pay that comparable arts and science graduates can command would have repercussions for many years to come. There is no visible sign that the government has decided that it wants a long term decline in numbers going into the scientific profession, indeed it has probably given little thought to the matter, but a settlement that leaves dissatisfaction in the scientific ranks will do much more than lead to temporary discontent.

Finally, and the greatest of the issues raised, does the government know what it wants of its own scientists?

It is obviously attractive to have all those scientific brains at one's disposal but are they always used to advantage? It would be unfair to ask for some sort of deal by which Civil Service science is reviewed in exchange for the coming pay award, but it does seem worthwhile to look again at the whole question of the government offering stable long term employment to such a large number of scientists. There is a feeling that the government holds too many scientists in an undemanding 'parking orbit' and industry and the universities should assume many of the piecemeal roles which the government has acquired. A fairly vigorous policy of hiving off over the next ten years, benevolently supervised by the government, may be no bad thing. And maybe to go with a reduced establishment, greater flexibility could go into pay negotiations. Collective bargaining removes much of the personal involvement that those with highly individual skill should have with their management. It would be good to see a greater devolution of negotiation on to management.

100 years ago



A SUPPLEMENTARY credit of 4,000*l.* has been voted by the Versailles National Assembly for paying a part of the expenses incurred by the observation of the Transit of Venus. Six members belonging to the ultra-clerical party have given a negative vote on a division. It is said they are not believers in the Copernican theory, and have no faith in the astronomical observations.

From *Nature*, 9, 452, April 9, 1874.



Seasonal rainfall forecasting in West Africa

Dr Derek Winstanley, now at the Ecological Systems Research Division, Environment Canada, Ottawa K1A 0H3, emphasises that seasonal rainfall forecasting is vital if early warnings are to be obtained of food shortages.

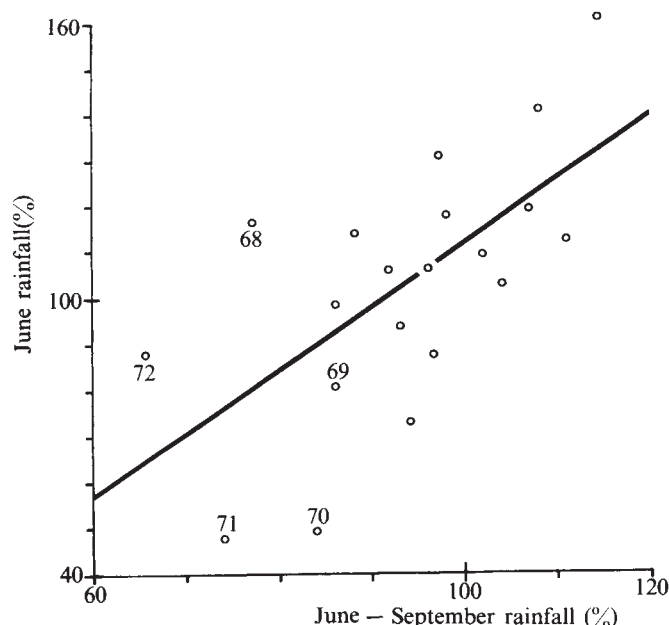


Fig. 1. Mean percentage of normal (1931–60) June rainfall at 59 stations between 10° and 20°N, from the Atlantic coast eastwards to Chad, plotted against the mean percentage of normal June–September rainfall for the 20 years 1953–72. The regression line has been derived by the least-squares method. Data sources are refs 5 and 6

Food production and the maintenance of the ecological balance in the semi-arid zone to the south of the Sahara depend on the rains which fall from June to September. For the past six years the rains have failed¹. Last year 3.5 million head of cattle worth £180 million perished, there was a grain shortage of 1 million tons and some 6 million people were placed on the brink of starvation. In order to avert disaster on an unprecedented scale in 1974, an increase in the already massive amount of international aid is essential. Clearly, successful seasonal rainfall forecasting could lead to an early assessment of food shortages.

I have taken 59 stations between 10° and 20°N, from the Atlantic coast to Chad, and have calculated the mean percentage of normal (1931–60) rainfall for June and for the wet season June–September each year during the period 1953–72. The mean June rainfall for the 59 stations is 124 mm and the mean amount for June–September is 636 mm, 88% of the mean annual total.

Figure 1 shows a highly significant correlation ($r = +0.63$) between June rainfall and the seasonal total. When June rainfall is below normal the total seasonal rainfall is also below normal.

There is clearly a strong tendency for anomalous rainfall patterns to persist throughout the wet season. This suggests an equally strong persistence of the large scale atmospheric circulation systems which control rainfall. The year 1968 was rather anomalous in that June rainfall was 17% above normal but the seasonal total was 23% below normal. This

was the first year of widespread rainfall deficiencies and it is possible that there was a rapid switchover of circulation types in about July of that year. Figure 1 shows that the subsequent dry years fit into the pattern.

The rainfall deficiency in this zone increases with latitude¹: at 18°N there has been a cumulative rainfall deficiency of 250–300% of normal since 1968, whereas at 10°N there has been only a small decrease in rainfall. I have previously suggested that the cause of this decrease in rainfall is a decrease in the northward extent and intensity of the ascending branch of the Hadley cell, concomitant with a weakening of the global atmospheric circulation^{1–3}. Taking the annual number of days of the westerly weather type over the British Isles as an indicator of the strength of the global atmospheric circulation⁴, it is apparent that for the past few years the global circulation has been weaker than at any time for about a century. The drought to the south of the Sahara thus represents an extreme situation but I have suggested that such droughts may become more frequent in the next 60 years³.

It is doubtful whether any nation can cope with a cumulative rainfall deficiency of 300% over 6 years. But the very fact that there are such large scale atmospheric controls on rainfall could provide a rational basis for integrated long term development. What must be determined are the maximum cumulative rainfall deficiencies that can be expected. It must then be decided what level of rainfall deficiencies can be tolerated, if the countries are to maintain some degree of self-support, either nationally or through some political or economic union. Development should then be encouraged in the more favourable areas, even though good rains may extend farther north in some years.

In 1973 world cereal stocks were reported to be at the lowest level for 20 years. Significantly, it was adverse weather in the Soviet Union in 1972 which led to the purchase of huge amounts of American grain; this, probably more than any other single factor, led to the depletion of world grain stocks and the escalation of world food prices. With depleted world cereal stocks it is not easy to guarantee supplies for major disaster relief programmes. Assuming that supplies are available on the international market, the high prices cripple the economies of the drought and flood-affected developing countries; in the case of India, the high cost of food imports has also necessitated the curtailment of the family planning programme.

The main development constraints in this zone are surely climatological, but the task of development seems to be beyond the individual capabilities of the governments. In any future development plans there are three points to be taken into consideration. First, large scale atmospheric circulations exert a unifying influence right across this zone, such that there will again be years when the rains fail over some 5 million square kilometres. Second, the atmospheric controls on rainfall are such that percentage deficiencies, and surpluses, will always increase with latitude. Third, climatic fluctuations in this zone are closely related to climatic fluctuations in other parts of the world, so that in some years there are likely to be synchronous food shortages in different regions which could affect world food stocks.

¹ Winstanley, D., *Bird Study* (in the press).

² Winstanley, D., *Nature*, **243**, 464–465 (1973).

³ Winstanley, D., *Nature*, **245**, 190–194 (1973).

⁴ Lamb, H. H., *Geophys. Mem. No. 116* (Meteorological Office, Bracknell, 1972).

⁵ *World Weather Records* (US Department of Commerce, Washington DC, 1967).

⁶ *Monthly Climatic Data for the World* (Environmental Data Service, NOAA, US Department of Commerce, Washington DC, 1961–72).

international news

Steel on steel for British rail

John Wilson

BRITISH RAIL (BR) will use steel wheels on steel rails to drive and support inter-city passenger trains of the 1980s, travelling at speeds up to 300 km h^{-1} (188 mph). Mr Mike Newman, in charge of BR's Advanced Passenger Train (APT) project, says that no existing linear motor can compete with present conventional electric units in efficiency and believes that the development of a magnetic suspension system with its costly new track is unnecessary for this sort of speed. His opinion conflicts with that of the Commission of the European Communities which recently invited manufacturers of high-power linear motors to Brussels to discuss cooperation in the development of just such a magnetically suspended, linear-motor driven system.

An experimental APT has already started a three year development programme by travelling at 200 km h^{-1} (125 mph) on a test track near the Derby works. This experimental train consists of a power car at each end separated by two trailer cars only, but commercial versions should propel about seven trailer cars. The whole articulated unit is highly streamlined and has a very low cross sectional area, roughly that of Concorde. The final versions will be built as very light semi-monocoque stress skin structures, a technique borrowed from the aircraft industry, and indeed the whole unit resembles an aircraft rather than a train in the design of the interior equipment and accessory systems.

The experimental APT is powered by conventional gas turbines, for the success of the British APT lies not in any revolutionary power plant but in its novel suspension system. Dynamicists at the Railway Technical Centre in Derby were the first to work out the complex mathematics of the ordinary railway wheelset (two flanged wheels rigidly attached to a live axle). They discovered that by altering the cone angles and tyre profiles of the wheels they could make the set roll with a minimum of oscillation. This allows the APT to be run at speeds of up to 250 km h^{-1} (155 mph) even on existing track and to negotiate main line curves



British Rail's APT: running on the same lines into the eighties

50% faster than conventional trains.

Greater speeds than these are possible with APT variants using more powerful engines, but considerable modification of the track is necessary. Mr Newman notes, however, that any improvement made in the track for the benefit of the APT would also benefit normal services. This is in contrast to other methods of high-speed travel where the new track is for the exclusive use of the new vehicle.

The keynote for BR's plans for the new train is the utilisation of existing assets. The brakes, for example provide double the usual performance expected for a normal train—and this in a vehicle of less than half the normal weight. For this reason the early APT's due to begin a trial service on the (London to Glasgow) West Coast main line in 1978 should be compatible with existing signalling equipment. Mr. Newman points out that by improving the track alone electric trains already in service could almost match the performance of the APTs. On a route such as London to Glasgow, which an APT with a speed of 250 km h^{-1} (155 mph) could travel in 3 hours 47 min, a conventional train could take as little as 3 hours 50 min—if all the necessary track improvements were made. Although it is worth noting that the APT could then chop another 37 minutes off this new improved route, the point is that these comprehensive track improvements would cost up to ten times more than an APT system.

The APT is an attempt to stretch an essentially Victorian railway system using 1960s technology. Any successor to the APT will have to be a completely new system. This is because the introduction of APTs is expected not only to compete more effectively with motor-

ways and airlines but also to generate considerable trade itself—to the point, in fact, where the system becomes saturated. It is this saturation, expected about the 1980's, not the need for higher speed, which will lead to the necessity to construct new track, and it is at this point that systems using linear motors, hovertrains and the rest begin to seem attractive.

Cancer research runs into trouble

Colin Norman, Washington

A COMMITTEE of biomedical scientists has delivered a stinging attack on a prominent and highly controversial cancer research programme supported by the US National Cancer Institute. The committee's criticisms and complaints cut deep into the philosophy underlying much of the loudly proclaimed federal war on cancer. Because they raise the basic objection that cancer research shouldn't be targeted and managed like a NASA-style operation to land men on the moon.

The research programme in question, the Virus-Cancer Program (which used to be called the Special Virus Cancer Program) is the epitome of the highly managed, centrally directed approach to cancer research. It now disburses more than 40 million dollars a year, entirely in contracts, to universities and industry for studies on cancer viruses and for the development of resources to support such research.

Ever since it was started ten years ago, with the specific approval of Congress, the Virus-Cancer Program (VCP) has been attacked for a variety of sins, chief of which is that it has been directed by a few people who have

amassed considerable power for themselves and that it has taken money away from potentially more productive research efforts.

In response to such complaints, the National Cancer Advisory Board, the body which oversees the work of the National Cancer Institute, set up a committee last year under the chairmanship of Dr Norton Zinder of Rockefeller University to take a look at how the programme is working. The committee turned in its final report to a board meeting in mid March and it said that virtually all the criticisms that have been raised against the VCP have some credence.

The committee's most basic objection is that "ignorance of the mechanism involved in the cancer process is so profound that it is difficult to be certain where to begin, much less to organize a focused attack". In other words, the basic knowledge just isn't available to begin a concentrated, highly directed research programme on cancer viruses, and the committee reckons that the best way to get the basic knowledge is to support a more broadly based research programme in the universities.

When congress passed the National Cancer Act two years ago, which set in train large increases in funding for cancer research, the implicit suggestion was that by shovelling more money into research and planning the effort with operations research techniques, answers would eventually emerge. A central feature of the act was, in fact, that a national cancer programme plan would be drawn up to provide a framework for the whole operation.

Since the committee's chief objection challenges the philosophy behind the Cancer Act, the report has had a surprisingly warm reception from the people who are in charge of the war on cancer. Dr Frank Rauscher jun., the director of the National Cancer Institute, said in an interview, for example, that the committee produced "a damn good report" and that the Cancer Institute is in fact in the process of righting most of the ills which it spotlights.

But the official enthusiasm for the report is not shared by the scientists who are operating the VCP. They sent a memorandum to the National Cancer Advisory Board in January charging that the committee's suggested improvements in the programme are "based on total destruction of the programme, and not on constructive modifications which could readily be implemented." They also charge, in private discussions, that the committee's criticisms were directed at a few individuals in the programme, on a rather personal basis.

In fact, the committee first presented its report to the National Cancer Advisory Board at a meeting last

November, but the board said that it was going to be so negative it ought to make its criticisms more specific and name names. But the report presented last month was unchanged from the earlier version. Asked why it had not been made more specific Dr Zinder said last week that it was meant to be an overview of the programme and he did not think it proper to single out individuals. He added that the members of the committee are willing to stand behind everything they said.

As for the committee's objections to the programme itself, they centre chiefly on the fact that the VCP is managed by a small group of scientists who have considerable power over the selection of contracts and the report suggests that the process by which contracts are reviewed is not very rigorous. In particular, the committee recommends that bidding for contracts should be more competitive, and that they should be reviewed by panels of scientists drawn from outside the National Cancer Institute.

Although it doesn't say it specifically, implicit in the committee's recommendations is that much of the money now distributed by the VCP through contracts should be made available to the academic community to support individual grants for projects suggested by researchers themselves and not by a handful of managers within the National Cancer Institute.

Zinder said last week that all the committee was trying to do is to "open the programme up to the academic community" by getting more outside people on the review committees and making more money available for investigator-initiated research projects.

The VCP scientists' memorandum to the board says, however, that the programme is not such a closed shop as the committee implies. The contracts support more than 2,000 individuals, including 600 professional scientists and 230 postgraduate and postdoctoral students, whose combined output of scientific papers in 1972 totalled 1,183. Moreover, between July 1971 and January 1974, the VCP received 149 unsolicited proposals for research projects and funded 22 of them, so there is a place for investigator-initiated research in the programme.

Be that as it may, several changes are being made within the National Cancer Institute as a whole and the VCP in particular which meet many of the objections raised by the review committee. For a start, the review process for contracts is being changed to allow greater participation by scientists from outside the National Cancer Institute. An overall advisory committee is being established for the VCP, which will consist entirely of outside scientists and which will advise the director of

the programme and the contract managers on the overall direction of the effort. And the committees which review individual contracts are being reconstituted so that outside scientists will be in the majority.

But the most far reaching change is that the National Cancer Institute is exploring a new funding mechanism which Rauscher calls programme grants. These will be broad based grants to university scientists which will not be encumbered by the controls and specific objectives that are attached to contracts. They will be used to support research designed to fill specific knowledge gaps. The idea is that programme grants would be peer reviewed by multidisciplinary study groups and they would run for perhaps three years.

Lamb's unit to the slaughter?

Allan Piper

THE existence of one of the two climatic research establishments in the western world, the Climatic Research Unit (CRU) at the University of East Anglia, is threatened by lack of financial support, at a time when the importance of climatic research is becoming increasingly clear.

Variations of climate cannot be prevented but they can be predicted with increasing reliability. Advance knowledge of an impending catastrophe—a drought, a flood, a severe winter—allows for careful planning which may often save lives and money. The CRU has been in existence for only two years and its director, Professor Hubert Lamb, says that the prospects for climatic research are excellent: "the plums are ripe for the picking, we only need someone to provide a stepladder".

The climatologists at the CRU are working towards the development of an increased understanding of the mechanisms and causes of climatic fluctuations. They have collected extensive data on weather and climatic behaviour going back to about 1200 AD and present day climatic developments are monitored on a global scale, often using observations from satellites. The knowledge acquired is used to supplement the present statistical techniques of ascertaining likely climatic trends and it is hoped that this will lead to increasingly reliable predictions.

Ever since the CRU was established in January 1972, it has had to operate under restrictive financial conditions. Initial grants came from the Nuffield Foundation, Shell International Petroleum, British Petroleum (BP), the Electricity Council and the Central Electricity Generating Board. These grants amount to less than £60,000 spread over five years and although

Professor Lamb considers them to be "very generous indeed", it is apparent that, compared with other research establishments, the unit is working on a sadly inadequate budget. Furthermore, an initially bad situation has been worsened by the effects of inflation, which eventually forced the withdrawal of the support from BP, although the other contributing bodies have been able to provide appropriate incremental adjustments. Now it is clear that by the end of 1975 the funds of the unit will almost certainly be largely exhausted. Even if the present grants are renewed, Professor Lamb can only remain cautiously optimistic about the likelihood of obtaining support from other organisations.

This is rather disappointing because there is a good case for increased financial investment in climatic research. Modern commercial and industrial techniques are geared to utilising to the limit any available resource, such as water, livestock or crops, and production methods are increasingly arranged to maximise profits. It is always likely that such finely adjusted projects will be vulnerable to any shift in climatic behaviour. Established patterns of production may become severely disrupted, often with disastrous consequences. A continuing decline in

rainfall can ruin not only intensive agricultural projects, but also sophisticated technological schemes such as hydroelectric power plants, or industrial processes which may be sensitive to such factors as atmospheric humidity. Results of recent research to assess differences in agricultural yield between a wet and a dry summer in New Zealand indicate that £2 million a year may be lost just on cattle in one small region alone. Investment in climatic research could save much of this: foreknowledge of climatic trends can facilitate advanced administrative planning, allowing otherwise catastrophic situations to be mitigated, if not avoided, by careful preparations of all necessary assistance.

The results which have so far been produced by the CRU, covering the past two years, are encouraging although not entirely accurate. According to Professor Lamb, however, the inaccuracies illustrate the problems of operating with restricted financial resources. Retrospective reappraisal of the data used reveals that the correct information had, in fact, been assimilated but it was misinterpreted and wrongly collated.

The errors arose chiefly because the unit is drastically understaffed, says Professor Lamb, and the consequent

pressures have not allowed for the assimilated data to be used to its best effect. As a result, the preliminary series of seasonal forecasts has had to be discontinued. There is both the room and the necessity for research workers at all levels of experience from graduate students upwards, and there has been no shortage of suitably qualified applicants; unfortunately the present financial stringency precludes any expansion. A ideally qualified, highly regarded applicant was recently refused a senior research post because of the lack of necessary funds.

Professor Lamb's disappointment is hardly surprising. With the unit left with a guaranteed existence of less than two years, he is not finding it easy to negotiate new sources of support although BP are considering the provision of renewed assistance. It is difficult to understand the reluctance of agricultural and industrial organisations—let alone governments—to grant the required funds, particularly in view of the enquiries already received by the CRU. These include requests from an industrial concern for information about the probability of severe winters occurring simultaneously in Europe, the USA, and Japan, and requests for forecasts from farming organisations in Norfolk, France and even Zambia. But Professor Lamb feels, not unreasonably it seems, that it is the international agencies which should provide substantial support for research which is "vital to the needs of mankind at the present time".

The position of the Natural Environment Research Council (NERC) is also interesting. It is the policy of the council to provide funds only for specified research projects; it will not allocate finances to assist in establishing a research group. For this reason the NERC did not provide support when the CRU was founded back in 1972. Today the NERC provides support for a research student at CRU but will not help to establish any new research projects. This policy contrasts markedly with that of the Medical Research Council (MRC) which will provide 'programme grants' to support "the particular situation in which a university has formally decided to develop a specific plan of study, and the Council agrees in the national interest to assist that development in that particular university."

It would be a pity if the existence of CRU were to be placed in jeopardy after such a short existence, at a time when the potentialities of the research work are about to be realised. The research staff have begun to establish liaisons all over the world, and it would be a great disservice to climatic research if these relationships were allowed to collapse.

US nerve gas plan under fire

A BRITISH expert on chemical and biological weapons has blasted the US Army's plans to develop a new generation of nerve gas weapons on the grounds that they will kill attempts to negotiate an international treaty banning production and stockpiling of chemical weapons. Julian Perry Robinson, a member of the Science Policy Research Unit at Sussex University who has been conducting a study of chemical and biological warfare for the Stockholm International Peace Research Institute, delivered his attack on the army's plans at a symposium organised by the American Chemical Society on April 1.

In September last year, the US Army announced that it is planning to produce new, "safe" nerve gas weapons at the Pine Bluff arsenal in Arkansas. The idea behind the weapons, which are called binaries, is that they will consist of two chemical components neither of which is lethal by itself, but which form a highly potent nerve agent when they are mixed together. The two chemicals would be loaded into separate compartments of a shell and allowed to mix only when the shell is safely on its way to the target.

The army is claiming that binary weapons have the advantage that they are safe to store, and transport, and it is planning to replace the immense

stockpiles of conventional nerve agents now stored in bases throughout the United States and abroad with binaries. These stockpiles have generated considerable public alarm, and opposition to the entire chemical weapons programme in the United States. The army is therefore hoping that its new safe weapons will be more acceptable.

Robinson points out, however, that a programme involving the production of binary weapons and the destruction of stockpiles of conventional nerve agents will cost American taxpayers as much as 2,000 million dollars.

But a more basic criticism of the programme is that if the United States now launches a massive new nerve gas programme, it will completely destroy the credibility of US intentions to negotiate seriously for international chemical weapons control. Talks have been taking place in Geneva for several years on a possible treaty banning use and stockpiling of such weapons, and although the United States position has not been exactly enthusiastic for such a treaty, at least it has shown willing to talk. Robinson argues, however, that "a decision to go ahead with binaries would almost certainly mean an end to the disarmament negotiations, and with them a prospect for improving US security to a far greater extent than binaries ever could".

Oil prices hamper oceanography

For most users of oil the recent price rises have simply meant paying more for the commodity and finding ways of living with the greater expense. For some, particularly those receiving fixed sums of money from the government, paying more is not possible without going bankrupt. In scientific research the worst affected are the oceanographers.

In Britain the Natural Environment Research Council (NERC) operates many of the research ships for universities and its laboratories. Largest of these are the *Bransfield*, *Discovery*, *Shackleton* and *Challenger*, although there are many smaller vessels some of which are the direct responsibility of individual universities. An official of the NERC pointed out that there would be an increase of "several hundred thousand pounds" in the 1974 fuel bill for these ships, this in a total budget for the operation of research vessels of only £1.5 million. As yet the NERC has not been able to extract any promises of extra money from the Treasury, so although marine operations are for the present carrying on as usual, within a few months there is a serious danger that cruises will have to be curtailed or cancelled.

One oceanographer expressed concern that the short term problems with oil prices should not affect long term decisions on building new oceanographic vessels. "It is clear that large scale capital expenditures are very vulnerable at times like these", he said, "but we cannot indefinitely postpone the renewal, let alone the expansion, of our oceanographic fleet". At present there are no new ships planned but there seems to be a general feeling both that some vessels cannot last much longer and that oceanography actually needs more ships to satisfy the demands of research workers. It looks, however, as if survival at the present level of activity is going to be the most that the NERC can aim for in the next year or two.

Soviet reactor accident: official

from our Soviet Correspondent

REPORTS of a serious accident to the BN-350 fast breeder reactor at Shevchenko on the Caspian Sea, although strenuously denied by official Soviet resources, do seem to have some foundation in fact, although the failures involved belong to the field of conventional, rather than nuclear, engineering.

According to Dr N. V. Krasnoyarov, interviewed when in London for an international conference on fast reactor power stations, three out of the six

generators of the Shevchenko station have, in fact, been taken out of service following faults in the interface of the secondary (sodium) and tertiary (water) circuits. In one case, the leakage of water into the sodium of the second circuit was observed at a very early stage and the generator was immediately closed down; in the other two cases, the water, reacting on the hot sodium with consequent liberation of hydrogen, caused a pressure buildup which resulted in the rupture of what Dr Krasnoyarov called "special safety membranes" (presumably bursting disks); the products of the reaction were conveyed to a special dump tank and the generators concerned were closed down.

All three faulty generators are now undergoing repair; in the meantime, the other three are being operated at below nominal capacity, giving a total output at the station of some 30% of its rated power. The deficit of power required for electricity generation and desalination is at present being supplied by the conventional oil-fired generators which the BN-350 was designed to replace but which were retained as a peak-demand backup. The primary (radioactive) sodium circuits were in no way involved.

The rumours that more serious damage had been detected by United States surveillance satellites seem to have grown by a snowball effect, from 'If

Better deal for polytechnics

THE 30 polytechnics in Britain are to get a better deal from the Science Research Council (SRC) as far as postgraduate studentships are concerned but the criteria governing the award of research grants will still favour university-type research. This move follows the report of the SRC's Polytechnics Working Group which has been considering the relationship between the SRC and the polytechnics since 1972.

The SRC is to put its new plans into operation through a new Committee on Postgraduate Training in the Polytechnics, which has been set up for a three-year period under the watchful eye of Dr A. H. Chilver, Vice-Chancellor of the Cranfield Institute of Technology. The Committee will be empowered to award advanced course studentships (usually lasting one year and aimed at the taught postgraduate course which the polytechnics have been encouraged to set up).

As to the award of research studentships, usually held for three years, the new committee will only advise other parts of the SRC "on any special factors to be taken into account in the award of research studentships to polytechnics". The SRC is keen that this should result in the channelling of more of

there had been a serious accident, the satellites would have detected it' to 'The satellites have detected a serious accident', and the July dating seems to have crept in by confusion with the date of commissioning of the station. The growth of the story was in no way quenched by the tone of the official denials. According to the Novosti agency, Academician Andranik Petrosyants, Chairman of the State Committee for Atomic Energy of the Soviet Union "emphatically denied" that anything untoward had occurred. Instead of the reasonable explanation offered by Dr Krasnoyarov, Academician Petrosyants simply maintained that "all this information does not correspond to reality" and that the report "is an invention and obviously pursues some other aims", being directed against Franco-Soviet cooperation in fast breeder reactors. "It is evidently of benefit to some people to try to do damage to that cooperation and set public opinion against that cooperation by inventing 'explosions'."

The tone of the denial seems inspired by considerations of prestige, rather than a desire to impart information. Perhaps if Academician Petrosyants had, in the name of the "cooperation" he advocates, been a little more forthcoming about what are after all relatively minor faults, his denial of a major disaster might have been found more credible.

the so-called CASE awards (Cooperative Awards in Science and Engineering) to polytechnics, thus giving a fillip to the cooperation between polytechnics and industry which the Department of Education and Science has always been keen on. At the end of 1972 students in polytechnics held 1.7% (104) of all SRC research studentships and 2.9% (45) of its advanced course studentships.

The volume of research in polytechnics is bound to increase in the next few years because by 1981 the government plans that there should be 180,000 undergraduate and postgraduate students in polytechnics on full-time or sandwich courses, compared with 68,000 in 1972. Clearly there will be a big demand for whatever the SRC has on offer.

Dr J. S. Bosworth, Director of Newcastle-upon-Tyne Polytechnic, said last week that the SRC's plans are certainly a good step in the right direction. He pointed out, however, that it is particularly important for polytechnics to get together with other interested parties and define just what research they are to do. In this way, he said, it should be possible to arrange a framework in which polytechnic-type research could be sensibly planned and carried out.

Lead in the environment

A RECENT report of rising levels of lead in the blood of people living near a large British motorway interchange has highlighted the problem of lead pollution through exhaust emission. Lead can enter the environment in many ways. Food is generally considered to be the main source of lead intake; it has been estimated that each person takes in about 200–250 micrograms of lead in food daily. Lead is also released during lead smelting and other industrial processes but these are now subject to strict controls and although there is still pollution this affects only small areas.

In 1972, about 90,000 tonnes of lead were added to petrol in the United Kingdom alone. Plans to reduce the level of lead in petrol to 0.45 grams per litre by 1975 have had to be shelved in view of the present oil crisis as producing high octane grades of petrol without the use of lead additives needs much more crude oil. At present the permitted maximum in the United Kingdom is 0.64 grams per litre.

The survey of blood lead levels of adults and children living around the Gravelly Hill interchange, 'Spaghetti Junction', was carried out by Birmingham City Council. Before the interchange opened in 1972 lead levels in residents' blood were on average 12.2 micrograms per 100 millilitres and last October had risen to an average of 16.6 micrograms. This January they showed a rise to an average level of 21.0 micrograms by the hospital analysis and 26.3 according to the Birmingham City Analyst. These discrepancies highlight one of the main problems in lead toxicology; the variability inherent in the methods used to measure blood lead levels.

The inference that is being drawn from the Birmingham report is that lead from traffic exhaust fumes is the cause of this rise in blood levels. The Birmingham Health Committee have expressed concern and are drawing the government's attention to the report. Although the average levels do not yet approach the accepted toxicity levels and are not above the upper limits for normality, a great deal of concern has been expressed over the past few years about the long term effects of exposure to subtoxic levels of lead especially in children. Also, there are bound to be individual higher than average levels.

The maximum acceptable level of lead in the blood of adult male workers exposed to lead for an eight hour day is considered to be 80 micrograms per 100 millilitres. For children the general consensus of opinion takes 36–40 micrograms as the upper limits of nor-

mality and considers 60 micrograms as cause for concern even though the child may not be showing any symptoms.

The whole question of the relation between the amount of lead in the atmosphere and its absorption into the body and link with blood lead levels is under intensive study at present. A study in 1972 by the Medical Research Council's Air Pollution Unit found that atmospheric levels of lead had risen in a busy London street from an average of 3.2 micrograms per cubic metre of air to 5.4 micrograms. A small survey of London taxi drivers during this time compared the blood lead levels of daytime with night-time drivers. Although



Spaghetti Junction: children at risk?

the daytime drivers were subjected to a higher level of exhaust pollution (monitored by the carbon monoxide effect on the blood) their blood lead levels were similar.

Much work on the size and aerodynamic properties of the lead particles given out in exhaust fumes is in progress. It has been estimated that about one-quarter of the lead content of petrol remains in the engine and the rest is given out in the exhaust as fine particles of lead compounds. Of this about half is thought to be deposited within a few hundred feet of the road but the finest particles may be carried considerable distances in the air. The fate of these particles in the lungs and how much is finally absorbed is also under study. The Atomic Energy Research Establishment at Harwell have just embarked on a study of the intake of radiolabelled lead from exhaust fumes to try and throw light on this problem. Earlier work on artificial aerosol lead intake indicated an uptake of about 40–50%, and more recent

work in other laboratories suggests that about 15–20% of atmospheric lead is absorbed. Some workers, however, think that this figure is too low.

At present, there are many surveys measuring atmospheric and blood levels but it is often difficult to correlate the results of such different surveys. In a recent report, the Central Unit on Environmental Pollution drew attention to the difficulty of correlating results from different surveys. The report names lead as one of six pollutants which need monitoring on a wider scale than before. They recommend that a network of 20 sites be established in various areas to monitor trace elements by a standard technique. At present, a survey of traffic fume pollution is underway in five major British cities, coordinated by the government's Warren Spring Laboratory.

Additionally, the Worshipful Company of Pewterers celebrated its 500th anniversary by setting up a trust fund to provide an income of £10,000 a year for a research group at the Institute of Neurology, enquiring into the effects of heavy metals on the central nervous system. The first Pewterers Fellow, Dr Jeremy Barlow, has pointed out that, for the time being, the group doesn't quite know what it's looking for, but that there are certain facts, like the increased incidence of mental retardation in children in recent years, and about the amount of lead in the environment, which may or may not be related to each other.

The situation is further complicated by the fact that some individuals have a greater tolerance of lead in the body than others; one man may have taken in lead to the extent that his blood contains 1,000 micrograms per millilitre, and appear to be no worse off for the experience, whereas another individual may exhibit the clinical symptoms of lead poisoning at levels which are accepted as relatively safe. What's more, the symptoms are easily confused with the general symptoms of what is known as 'modern living'—gut pains, insomnia, loss of appetite, and headaches, so that it's difficult to say that lead is specifically responsible whenever these symptoms occur.

Clearly a great deal of research has yet to be done before it is possible to say whether or not atmospheric pollution from car exhaust fumes is in any way responsible for higher levels of lead in the body, and that life, or health is endangered by the motor car. If we really want to be on the safe side in the meantime, it's possible to cut the lead content of petrol by producing high octane grades at great expense, or to stop using high-compression engines, which would mean that an awful lot of nice new cars would be made obsolete, at a stroke.

correspondence

Prices of scientific journals

SIR,—The risk of being misquoted from telephonic communications (me to *Times Higher Educational Supplement*, October 26, 1973) increase when the misquotation is quoted, for I am the Alec Henderson who figures in John Hall's article (*Nature*, February 15). One original misquotation was that the well known *IEEE Transactions* becomes the mythical *Transactions of the American Association of Electrical and Electronic Engineers*: but the desolating fact is that *IEEE Trans.*, having already gone up from £252 to £321 this year, has now gone up again to £411—a rise of 63% in a year. On the other hand, it is fair to point out that the quoted increase in price in *Current Contents: Life Sciences* applied only if one took a new and optional indexing service.

The rate of rise in journal prices is alarming. That of books has hitherto been somewhat less but some alarming trends are evident, particularly in marking up of in-print books in publishers' stocks. Recent correspondence with the American publisher of a work on polymerisation explained that the rise in the United Kingdom price of a book of 416 pages from around £16 to £24 was due to floating exchange rates, air-freight charges, warehousing and distribution costs and paper costs—all in the case of a book first published in 1972, not reprinted and listed at the original price in the publisher's catalogue dated 1973–74.

University library budgets in the United Kingdom as a whole were not keeping up with the combined effects of rising prices, diversification within universities and proliferation of literature even before Mr Barber's cuts in public spending. To these cuts, particularly to that in supplementation for price increases, they are particularly vulnerable because of the large proportion of their expenditure which goes on books and journals and subacademic staff (supplementation of academic salaries being safeguarded): they must therefore receive generous treatment from their universities if they are not to fall further behind in service to their readers. Librarians thus do not need to be urged by Professor Linnett and his co-signatories to "exercise the greatest reticence on subscriptions to new journals"; they already have to do so,

against the insistence of their scientists (and others) that exception must be made for the latest expensive journal which is just their thing.

If scientists can reduce the volume and cost of publication, whether by refraining from publishing or by compression, they will themselves reap the benefit of the greater accessibility of what is published since their libraries will be able to afford more of the available literature. If Mr Maxwell's plans for *Tetrahedron* will reduce by 80% its price (which I do not suggest is excessive) as well as its bulk, Bubbs, Humphreys, Anderson and their fellow librarians will be glad to join with Finniston, Hartnett, Kurti, Thompson and their fellow scientists in praising his "imaginative leadership".

ALEX. ANDERSON

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SIR,—The best way through the scientific prices jungle (*Nature*, 247, 417; 1974) is for scientists to give wholehearted support to librarians in their efforts to establish objective criteria of usefulness. It is possible to tell how frequently a title is cited in published papers (especially since the "Journal ranking package" has become available from the Institute for Scientific Information); it is possible to tell how often the title is photocopied, how often the title is borrowed on inter-library loan and how often it is being consulted in the library, if each volume and part has a consultation slip. These may not be very good measures of usefulness but they are much better than no measures at all. All that is lacking is a firm decision on the part of the scientific community that:

- No journal should be bought until the cost of borrowing it from the British Lending Library exceeds the cost of buying and storing it on site.
- No journal held on site should be maintained unless objective evidence can be produced that it is being used.

I have little doubt that this policy, if strongly backed, would be a major factor both in improving the quality of science journals and in controlling the prices jungle, to the benefit of library users and librarians alike.

P. G. PEACOCK

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Government scientists' pay

SIR,—It is now widely known that government scientists have been in dispute with the Civil Service Department for over three years because the department has been unwilling to agree on any reasonable criteria for determining the level of scientists' pay. As a result of this long and bitter dispute, government scientists have seen their pay fall, relative to colleagues with whom previously they enjoyed parity, by 20% and in some cases by even more. Since the government is the major employer of scientists in the United Kingdom, this decline in the pay of government scientists has led to the whole of the science area becoming a depressed industry.

What does not seem to be so widely appreciated is the likely long term effect. The status and success of the United Kingdom in the world have been founded to a large degree on the inventiveness of its people and their achievements in the field of science and technology. What is true of our history seems to us even more true today. Yet, at this critical time, we find that 6,000 places for scientists in the universities are vacant, that fewer young people are studying science at school and that scientists are leaving their chosen careers at an alarming rate to take up other occupations.

Since a decision to adopt a scientific career is often taken at the age of 15, in effect it takes six years to train a graduate and nine to train a PhD. Thus the ultimate effect will be to damage the growth of all science-based industries for a decade or more. We believe this is too high a price to pay for an unnecessary dispute.

As responsible members of the scientific community, we look forward to a speedy resolution of the present dispute and to a time when it will again be possible to urge young people to take up science as a socially desirable and reasonably rewarded profession.

J. A. GIBSON
P. HAWTIN

The Institution of Professional Civil Servants,
Atomic Energy Branch,
Harwell Section,
AERE, Harwell,
Didcot, Berkshire, UK

news and views

Chinese granites and rapid seafloor spreading

THE attention of geologists and geophysicists, for a long time focused on land masses, switched to the oceans in the mid-nineteen sixties when it became clear that the movement of continents could only be understood in terms of processes occurring in ocean basins. Now that there is a fair consensus on submarine geology and geophysics, attention is switching back to continents where there are still some very difficult problems to solve but where the insights of the past ten years can help. Two new papers exemplify the questions that are being asked.

Plate tectonics is a good framework for running the geological clock backwards for the most recent 100 million years, as the magnetic record is well preserved for this period in oceanic crust. Attempts to deduce the disposition of the continents at earlier dates, however, can be less certain because much of the ocean crust formed then has been consumed at island arcs. As with Watergate, the magnetic tape recording is missing, so other sources of evidence must be sought on land. This Jahn does later in this week's *Nature* in using newly determined ages of rocks in south-east China to study a period of exceptionally vigorous seafloor spreading about 100 million years ago. Correlations which he tries to establish within the time of magnetic records may then be extended to earlier times of intensive granitisation when there is no seafloor record.

By a coincidence it is the same area which is the subject of the other paper which originated in the Department of Geology, Nanking University (*Scientia sin.*, 17, 55-72; 1974). Better knowledge of tectonics means better knowledge of mineral deposits. The cycles of tectonic activity in south-east China have led to rich sources of ore, and it is of great importance to know which regions are richest and thus can be most easily exploited.

The Nanking group have dated the distinct tectonic zones of granites that run NE-SW in south-east China. In general the older the rocks the further inland they are. Each zone is about 100 km wide and characteristic ages are 900, 480, 200 and 100 million years, the last being called the Yenshan tectonic zone and forming the coastal belt. The authors indicate the following series of events for each cycle of tectonism: subsidence, sedimentation often with volcanism, orogenic movements, granitisation, regional uplift, subsequent crustal movement and small scale granitisation. They go on to observe "the further development of the Himalayan orogenic zone in our Taiwan province and the presence of island arcs and a submarine trench east of Taiwan indicate that the same tectonic processes have been going on at present".

Analysis of metallogenetic relations and mineralisation capacity of the granite of different zones necessarily produces complex results, but there are some very striking unifying factors. They are best seen in multiple-aged composite granite bodies where several tectonic cycles have left their mark. Ore forming elements such as beryllium, tungsten, tin and niobium are progressively concentrated in the younger bodies. Yenshan rocks contain beryllium, niobium,

tantalum and scandium in workable deposits. The authors conclude that it is the recurrence of granitisation rather than its occurrence on one occasion which leads to significant metallic deposits.

But what are these cyclic tectonic events? It is this question to which Jahn's paper contributes, as it looks at the Yenshan orogeny. Jahn is limited to samples from the offshore islands; these give ages of around 100 million years. Thus the Yenshan orogeny occurs simultaneously with a postulated time of very rapid (up to five times normal) seafloor spreading, established from marine magnetic data. What is more, many others have observed vigorous activity around this period in circum-Pacific regions. Maybe there is a correlation between rapid spreading and intensity of thermal episodes, caused perhaps by an enormous increase in viscous heating as old plate descends into the upper mantle. Jahn is cautious about such a correlation, but the evidence is certainly persuasive.

Some interesting problems are raised by these two papers. If this correlation is correct, is it generally correct to associate all previous Chinese orogenies with times of rapid seafloor spreading (for which it has been already noted there is no magnetic record)? Was rapid spreading a world-wide phenomenon, and how was it caused? Will the next pulse of rapid spreading convert the continental shelf off China into another tectonic belt? These are good questions for bright research students.

D.D.

Ghost neutrinos emerge from the mathematics

It is a remarkable fact that even after two generations, the investigation of solutions to Einstein's famous field equations of general relativity is still very much in its infancy. There are indeed very few exact solutions available, and some of those which are have very bizarre properties. One recent solution, published by Davis and Ray of Clemson University in *Physical Review D* (9, 331; 1974) is a distinctive mathematical novelty.

To understand the implications of this new result, first recall that the gravitational field equations of general relativity are a prescription for calculating the curvature of space-time from the mass-energy content of the Universe; that is, the equations relate the geometry of space-time to the physics of matter and energy. Thus for a given distribution of matter say, the geometry of space-time in its vicinity may in principle be deduced. In practice, the equations are prohibitively difficult to solve in all but the simplest cases. One of the earliest solutions, due to Schwarzschild, give the geometry around a spherically symmetric object such as a star. The gravitational bending of light by the Sun testifies to the curved nature of space-time in its vicinity and Schwarzschild's solution gives the correct amount of curvature. Just as the geometry is determined by the material content of space, so in turn the behaviour of mass-energy depends on the geometry. There is clearly a problem of self consistency here.

Among the more elusive of the elementary particles is the neutrino, the particle produced in, among other circumstances, the β decay of a radioactive nucleus. Neutrinos have no mass or charge, but they do carry spin, and are

rather like massless, chargeless electrons. They interact only very weakly with ordinary matter so that most would pass right through the Earth without stopping. Nevertheless, neutrinos cannot be ignored in general relativity, for it is widely believed that they are the most numerous of all particles in the Universe, having emerged in copious quantities out of the 'big bang'. The motion of the neutrinos, which is always at the speed of light, is described by the Dirac equation. A self-consistent solution of the combined Einstein-Dirac equation therefore supplies a possible space-time geometry consistent with a possible current of neutrinos. It is just such a solution which Davis and Ray have found, but one with rather strange properties.

The precise mathematical quantity which describes the curvature of space-time is known as the Ricci tensor R_{jk} , which is a 4×4 array of numbers defined at each point of space-time. Davis and Ray choose a geometry which is restricted partly by having plane symmetry around the x axis and it turns out that for this case only the diagonal components R_{ii} of the array are nonzero. On the other hand, the physical quantity which curves space-time is the stress-energy, which may also be written as a 4×4 array of numbers at each point. Using the Dirac equation in their selected geometry, Davis and Ray then proceed to calculate this second tensor for the neutrinos. All but two of the components are immediately seen to be zero and of the two that are nonzero neither is along the diagonal. Now in the neutrino case Einstein's field equations simply reduce to the equality of these two tensors (apart from a multiplicative constant including Newton's constant of gravitation, G). Consequently, the two remaining off-diagonal terms of the stress-energy tensor must also be zero, because all the off-diagonal R_{jk} terms are. So it turns out that the whole stress-energy tensor for the neutrinos in this particular space-time vanishes. Einstein's equations are exactly the same as they would be for empty space. And in fact, these very empty space equations were already solved many years ago by Taub.

At first sight it might seem that Davis and Ray had somehow put the neutrinos in and then taken them out again. This is, however, not so. That the neutrinos are still there is demonstrated by the fact that the neutrino current is explicitly nonzero. In principle it would be possible to detect such a current in a β -decay experiment.

Of course there is no suggestion that the solution described here in any way corresponds to the situation actually found in the real Universe. Nevertheless, it is somewhat surprising to find that the general theory of relativity admits at all the possibility of a current of particles which may produce physical effects, and yet make no additional contribution to the gravitational field. Because of this property Davis and Ray have invented the term 'ghost neutrinos'. It would be interesting to know if ghost particles may exist under a wider range of circumstances. P.C.W.D.

Amplification of VLF radio signals

VERY low frequency (VLF) radio waves have several interesting properties. In the ionosphere they split into two waves; one is strongly reflected and is of immense importance for long distance communications, the other, the extraordinary or 'whistler' wave, travels through the magnetosphere weakly guided by the Earth's magnetic field.

Whistlers, from which this type of propagation gets its name, were probably first detected on telephone lines in Austria towards the end of the last century, but the first published examples were those observed during the First World War by Barkhausen, a German radio engineer,

as he listened in on allied telephone conversations.

The whistler is a falling tone, the frequencies (f) of which Eckersley found to have travel times which were proportional to $1/\sqrt{f}$. It was not until the early 1950s that Storey, working in Ratcliffe's group at Cambridge, discovered that whistlers were in fact the dispersed radio energy from atmospherics generated in thunder storms. Storey showed theoretically that the VLF electromagnetic energy would propagate along the Earth's magnetic field direction and be received in the opposite hemisphere as a falling tone with the right frequency-time characteristics. The whistlers which were received at Cambridge travelled along paths which carried them to more than 10,000 km from the Earth and showed, for the first time, that at this distance there were an appreciable number of electrons and ions ($\sim 500 \text{ cm}^{-3}$) whereas, formerly it had been considered virtually void.

This discovery triggered off considerable research activity and the number of natural VLF phenomena was multiplied; efforts to explain them lead to a greater understanding of the plasma and energetic particles trapped in the Earth's magnetic field. Many of the generation mechanisms proposed for natural emissions involve growth or amplification of electromagnetic wave by means of resonant interaction with energetic particles. A recent experiment carried out by McPherson, Koons and Dazey of the Aerospace Corporation and by Dowden, Amon and Thomson of the University of Otago (see later in this issue) has revealed that the signal from ground transmitters may also be amplified in the magnetospheric plasma. A signal at 6.8 kHz from a transmitter in Alaska was received in New Zealand at a station which was situated close to the geomagnetically conjugate point. The interesting feature of the experiment was that during the transmission the received signal strength increased, for a short period, from below noise level to a maximum of $35 \mu\text{V m}^{-1}$, before fading again. This maximum value represented a signal 27 dB greater than that calculated theoretically and the authors attribute this to amplification in the magnetosphere.

One of the chief difficulties in this type of experiment is to radiate adequate power at these long wavelengths ($\sim 200 \text{ km}$) and this was neatly overcome by these experimenters by using a monopole held vertical by a balloon at an altitude of between 1 and 1.5 km. Even with this arrangement the total radiated power was only 13 W.

It is interesting to note that McNeill, also in New Zealand, reported (*J. geophys. Res.*, **73**, 6860) as early as 1968 evidence of amplification of signals from the Seattle transmitter (NPG) at the rather higher frequency of 18.6 kHz. This effect, which amounted to an amplification of about 10 dB, was noted by McNeill during studies of frequency shifting mechanisms in the magnetosphere.

It is now generally agreed that to receive VLF signals in the conjugate region the energy must be guided by ducts of enhanced ionisation (or perhaps troughs). It seems from work carried out mainly at Stanford that in the absence of such enhancements the wave energy is not guided sufficiently closely to the Earth's magnetic field to produce an appreciable signal in the opposite hemisphere and in some cases the energy may even be reflected or absorbed. McPherson *et al.* and Dowden *et al.* identify the duct in which the propagation probably occurred from whistlers which were observed during the transmission period. All the whistlers observed were shown, by a now well established technique, to have travelled in a single duct and the authors quite reasonably assumed that the energy from their transmitter travelled in the same duct.

Though this experiment was performed only at 6.8 kHz, the transmitter is capable of working at frequencies up to 21 kHz and future research should show how this amplification depends on frequency, the time of day, transmitted power and the conditions prevailing in the magnetosphere.

Another amplification or wave growth process which has received attention recently is the transverse resonance instability. In this instability, which leads to the generation and growth of VLF waves in the magnetosphere, the high energy tail of the trapped particle distribution is unstable if the pitch angle distribution is anisotropic. The process is thought to explain ELF and VLF hiss at medium latitudes. Kennel and Petschek (*J. geophys. Res.*, **72**, 1; 1966) originally showed that particles with energies "in excess of the magnetic energy for particles ($B^2/8\pi N$, where B is the magnetic field strength and N is the ambient plasma density) would be unstable to wave growth and that the instability would lead to particle precipitation and a limit to the fluxes of particles trapped in the magnetosphere. This limit to the numbers of trapped particles was considered important and in agreement with satellite observations. The existence of this limit is now questioned by Etcheto and her colleagues (*J. geophys. Res.*, **78**, 8150; 1973) who have established a self-consistent solution to the equations governing the wave growth and particle diffusion. This solution suggests that there is strictly no 'limiting flux' and that the equilibrium flux of trapped particles depends on the rate of injection of particles into the trapping region of the magnetosphere.

The amplifying and wave growth processes are clearly of increasing importance in this field for they contribute in different ways to both the strength of the transmitted whistler mode signal and the background noise which interferes with its reception.

A.R.W.H.

Aspirin, Boston and Little Rock

from a Correspondent

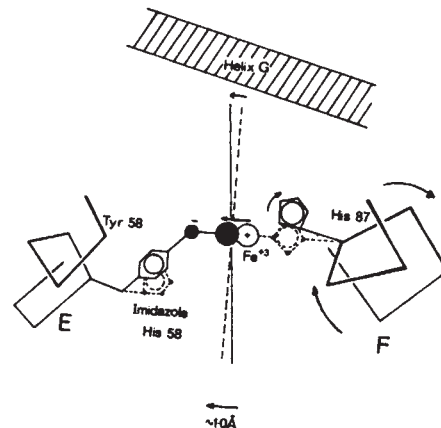
It may be asserted with some confidence that the genetically determined haemoglobin variants provide the most impressive roll-call of place names from all over the world, from Saskatoon to Sydney, and from Toronto to Tongariki. The appearance of aspirin in the title of this column, however, calls initial attention to a paper which appears to dispose of yet another proposed therapy for sickle cell anaemia. Starting from the established transfer, both *in vitro* and *in vivo*, of the acetyl group of aspirin to an ϵ -NH₂ group of lysine of serum albumin, Klotz and Tamm in 1973 had reported a similar transfer to haemoglobin, accompanied by a substantial increase in oxygen affinity. It was considered that this shift might be due to acetylation of the NH₂ terminal valines; a similar shift is observed following their carbamyla-

tion by cyanate, which is being intensively studied as a possible drug for the inhibition of sickling of sickle-cell anaemia red cells *in vivo* (see *Nature new Biol.*, **242**, 33; 1973).

De Furia, Cerami, Bunn, Lu and Peterson (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3707; 1973) have re-examined the effect of aspirin on the oxygen affinity of haemoglobin. They find that although the acetyl group is indeed transferred, both *in vitro* and *in vivo*, there is no effect on the oxygen equilibrium of haemoglobin, either in solution or in intact red cells. Furthermore, patients on long-term aspirin therapy show no shift in the oxygen affinity of their blood. They also failed to observe any inhibition of *in vitro* sickling of deoxygenated sickle cell erythrocytes previously incubated with aspirin, and conclude that this drug is of no value in the treatment of sickle cell disease.

The gelation of sickle cell haemoglobin itself has been the subject of theoretical study. Minton (*J. molec. Biol.*, **82**, 483; 1974) has devised a thermodynamic model to account for the macroscopic solution properties of Hb-S in terms of microscopic structure and interactions. In this model step 1, the formation of a rod-like microtubular array, is treated as being equivalent to a precipitation. Step 2, the formation of the nematic gel phase, is treated as an isotropically-aligned transition in a suspension of interacting rod-like particles. By combining the two steps a qualitative temperature-composition phase diagram is obtained which provides a unified interpretation of many of the observed properties of Hb-S solutions.

In Hb-M(Boston) the distal histidines in the haem pockets of the α subunits are replaced by tyrosines; His E7(58) $\rightarrow$ Tyr. The iron atoms of the abnormal α subunits remain in the ferric state to give a natural valency hybrid α_2^{+M} Boston β_2^{deoxy} . In heterozygotes for Hb-M(Boston) there is methaemoglobinemia because of the ferric α chains and cyanosis because of the low oxygen affinity of the normal β chains. Pulsinelli, Perutz and Nagel (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3870; 1973) have now studied this variant by X-ray analysis. A difference electron density map of deoxy Hb-M(Boston) minus deoxy Hb-A shows that the ferric iron atom in the α subunit coordinated to the four nitrogens of the porphyrin and the phenolate group of the tyrosine. The normal bond to the proximal histidine F8 (87) is absent and the iron, which is therefore five-coordinate, lies on the distal side of the porphyrin ring (see figure). The rupture of this bond causes the F helix to shorten slightly "like a spring released from a tension that had kept it slightly un-



Stereochemical changes in the haem pocket of the α subunit on going from deoxy Hb to Hb-M(Boston) (from Pulsinelli *et al.*).

coiled". The resulting changes in tertiary structure of the α subunits stabilizes the deoxy or T structure of the haemoglobin tetramer, which lowers the oxygen affinity of the normal β subunits. Thus the clinical effects and many of the abnormal properties of Hb-M(Boston) can now be accounted for in structural terms.

Haemoglobin Little Rock is a high oxygen affinity variant which is unique in having normal Bohr effect and haem-haem interactions. Collaboration between Bare, Alben and Bromberg (Ohio), Jones and Brimhall (Oregon) and Padilla (Little Rock) (*J. biol. Chem.*, **249**, 773; 1974), has now established that the alteration in sequence is β His H21 (143) $\rightarrow$ Gln. Since the β 143 histidine is an important binding site for the allosteric effector 2, 3-diphosphoglycerate, they have studied the effect of DPG on the oxygen affinity of Hb-Little Rock. They find that the DPG dissociation constant for deoxy Hb-Little Rock is about twice that for deoxy Hb-A, and the corresponding value for the oxy form about four times that of oxy Hb-A. They conclude, however, that the higher oxygen affinity of Hb-Little Rock cannot be explained solely in terms of reduced allosteric interaction with DPG, and that the Gln residues at β H21(143) must have a further specific effect on the oxygenation equilibrium.

Immunodepression in protozoan infection

from our Parasitology Correspondent

ANY ideas that patients with malaria or sleeping sickness readily succumb to other infections because of malnutrition associated with the disease are losing ground as more evidence accumulates to suggest that the immune response to other antigens is depressed

during protozoan infections. As long ago as 1962 it was realised that children suffering from malaria had diminished responses to tetanus toxoid (McGregor and Barr, *Trans. R. Soc. trop. Med. Hyg.*, **56**, 364; 1962) but the reasons for this were not investigated for another decade. Under experimental conditions, mice infected with malaria respond less well to tetanus toxoid than do controls (Voller, Gall and Manawadu, *Z. Tropenmed. Parasit.*, **23**, 152; 1972) and this has been confirmed in children (Greenwood *et al.*, *Lancet*, **i**, 169; 1972). The mouse malaria model has provided considerable information on immunodepression during malaria infections and the antigens to which the immune response is diminished include not only standard preparations but also infectious agents such as Moloney leukaemia virus (Bamford and Wedderburn, *Nature*, **242**, 471; 1973).

In laboratory models of trypanosomiasis, Goodwin and his colleagues (*Brit. J. exp. Path.*, **53**, 40; 1972) found that infected mice and rabbits had reduced abilities to produce antibodies against sheep red blood cells and Urquhart and his associates (*Trans. R. Soc. trop. Hyg.*, **66**, 342; 1972) found that the rejection of the nematode worm *Nippostrongylus brasiliensis* was impaired in rats with trypanosomiasis. Immunodepression is less easy to test in human trypanosomiasis, but Greenwood, Whittle and Molyneux (*Trans. R. Soc. trop. Med. Hyg.*, **67**, 846; 1973) were able to examine 38 patients with Gambian sleeping sickness using skin tests antigens and *Salmonella typhi* vaccine. The humoral and cellular responses were both less in these patients than in 43 uninfected controls. None of the patients was suffering from malnutrition.

Immunodepression has also been shown to be associated with experimental toxoplasmosis by Strickland and his associates (*J. infect. Dis.*, **126**, 54; 1972). Infected mice were less able to mount an immune response against malaria than controls.

A general pattern seems to be emerging in which immunodepression is a constant feature of protozoan infections. The effects need not necessarily always be harmful for autoimmune diseases may be prevented in infected animals as in the cases of allergic encephalomyelitis and trypanosomiasis (Allt, *et al.*, *Nature*, **223**, 197; 1971) and spontaneous autoimmune disease of New Zealand mice and malaria (Greenwood and Voller, *Clin. exp. Immunol.*, **7**, 805; 1970).

The mechanisms of immunodepression are still obscure despite the wealth of examples available for study. Antigenic competition is thought to be one explanation, but the possibility of

specific interference with the function of T cells is receiving attention (Bamford and Wedderburn, *Nature*, **242**, 471; 1973). The problem deserves urgent attention because of the possible danger involved in vaccinating children and others infected with a range of tropical diseases. The relations between malaria and Burkitt's lymphoma have already received considerable attention (Ziegler, *et al.*, *Trans. R. Soc. trop. Med. Hyg.*, **66**, 285; 1972).

A curious difference between parasitologists and virologists exists in this field. Parasitologists talk of immunosuppression and virologists of immunodepression. The experimental evidence points to depression rather than suppression; the virologists' term is preferable and they used it first.

Interpreting heat flow

from our Geomagnetism Correspondent

IN the sense that heat plays a part, and very often a crucial part in all internal terrestrial phenomena, study of the Earth's thermal processes may be regarded as the most important of all branches of geophysics. But by the same token it is a difficult subject to come to terms with. Heat sources and sinks abound; yet data are sparse, difficult to determine and even more difficult to interpret. The very complexity of the situation militates against the drawing of significant conclusions; and as two new reports demonstrate, heat flow workers, through no fault of their own, can easily appear as the thermal counterparts of the Ancient Mariner who found "water, water everywhere, nor any drop to drink".

For example, Sass *et al.* (*Earth Planet. Sci. Lett.*, **21**, 134; 1974) have taken advantage of geological investigations into possible sea level canal routes to measure heat flows in eastern Panama and north-western Colombia, the area where the Cocos, Nasca and Caribbean plates interact. There are, of course, few thermal situations more complex than the zones in which three lithospheric plates meet, for in such regions heat sources and sinks lie in close proximity to each other. A relatively cold lithospheric plate being thrust under an adjacent plate acts as a sink, but shearing along the boundaries between plates may produce frictional heating and thus a contribution to measured heat flow. Moreover, if in addition partial melting occurs, surface heat flow may be increased by the upward penetration of magma. These processes occur where two plates meet; at triple points they are even more intermingled.

In the present case Sass and his colleagues have obtained twelve new heat

flow values—three from the Canal Zone and three from each of three prospective sea level canal routes (routes 10, 17 and 25). In the vicinity of route 10, which lies 50–100 km west of the present Canal Zone, the average heat flow is high at $1.77 \mu\text{cal cm}^{-2}\text{s}^{-1}$ (h.f.u.). Previous work has shown that heat flow is also high south of Panama where it is associated with relatively young oceanic crust near the East Pacific Rise and the Galapagos Rift zone and with recent tectonic activity in the Panama Basin. The new land values, however, may be explained in terms of both the plate tectonic situation and the region's geological features. Sass *et al.* thus suggest that the high heat flow from route 10 may represent the easterly limit of a high heat flow zone associated with the Cainozoic volcanic centres which extend westwards into Costa Rica and Nicaragua.

Route 17, which lies 100–150 km south-east of the Canal Zone, and the Canal Zone itself are associated with heat flow values in the range 0.94–1.40 h.f.u. These are consistent with the low-to-normal values previously found at sea in the Caribbean plate to the north where, because of the oceanic character of the basement, it seems probable that most of the observed heat comes from mantle sources. The maximum contribution from the crust is thus probably no higher than 0.1–0.2 h.f.u. Some 100–150 km further south-east, along route 25, however, heat flow is even lower (0.66–0.70 h.f.u.). Because the average here (0.68 h.f.u.) is lower than even the theoretical 'mantle' heat flow found by Roy *et al.* (in *The Nature of the Solid Earth*, McGraw-Hill, 1972) for the Palaeozoic craton of North America, the observed surface heat flow presumably reflects the heat sink associated with the underthrusting Nasca plate.

In summary then, the new heat flow values obtained by Sass and his colleagues appear to be consistent generally with plate tectonics and surface geology even though they do little by themselves to sort out the complex interactions giving rise to them. By contrast, the analyses reported by Negi *et al.* (*Earth Planet. Sci. Lett.*, **21**, 143; 1974) seem to be inconsistent even with previous work in the same field. Some years ago Horai and Nur (*J. geophys. Res.*, **75**, 1985; 1970) carried out correlation and regression analyses of terrestrial heat flow (q) and thermal conductivity (k) which suggested that for many continental areas q and k are not independent. In fact, q correlated positively with k , a phenomenon which Horai and Nur put down to the fact that, in an inhomogeneous crust, heat flow will converge where the crust is more conductive. They further proposed that since q is proportional to heat production (A), k must also be proportional to A —a conclusion whose

necessity was later disputed by Naidu (*J. geophys. Res.*, **76**, 3426; 1971) but reaffirmed by Horai and Nur (*J. geophys. Res.*, **76**, 3428; 1971). A further conflict then came to light when it became clear that although Horai and Nur had found that the positive q - k correlation applied to the Indian peninsular shield, Gupta and Rao (*Bull. natn. Geophys. Res. Inst.*, **8**, 87; 1970) had already found no such correlation in the Indian Precambrian crystalline regions.

Continuing along similar lines, and to make matters even more confused, Negi and his coworkers have found that for all Indian sedimentary basins q correlates with k negatively. On the other hand, a positive correlation between heat flow and geothermal gradient (G) agrees with that found by Gupta and Rao for Indian Precambrian crystalline areas. Finally, heat flow is found to correlate negatively with crustal thickness.

What does it all mean? Negi *et al.* conclude that for sedimentary basins, at least, the negative correlation between q and k implies very little heat generation in the crust; in other words, most of the observed heat comes from below. The observed correlations between q , k , G and crustal thickness are often presumed to be due to variations in crustal thickness which produce a variable shielding effect on subcrustal heat. These are rather mundane conclusions to emerge from so much work; and it is difficult to judge their importance. Clearly, however, the parameters involved are very basic to the whole question of variations in heat flow; and so it would be helpful if someone would put all the data together and, if possible, explain just what is to be made of them.

Semiconductors in the human body?

from our
Solid State Physics Correspondent

It was realised about 20 years ago that the production of energy from oxygen in the cell mitochondrion was possibly the result of direct electronic transport through haemoproteins rather than the interaction of mobile ions through an aqueous medium (see, for example, Szent-Györgyi, *Discuss. Faraday Soc.*, **27**, 111; 1959; Carden and Eley, *ibid.*, 115). It was thus necessary to think about a sort of wet solid-state physics which could embrace reasonably high rates of transport of free electrons or holes through biological solids, especially through certain haemoproteins involved in respiration. Szent-Györgyi proposed a theory that a suitable conduction band could be produced by orbital overlaps in amino acid chains and one could predict from

this theory that proteins might achieve conductivities in the semiconductor range. The theory was conceptually very attractive but was not widely taken up, largely because it was not at the time susceptible of proof, nor had any investigation ever revealed a way of making analogous but simpler organic materials conduct to the level required.

Now, at least, one biological material has been shown to have a strikingly large conductivity when correctly excited. McGinness, Corry and Proctor, of the University of Texas Cancer Center, Houston, report in *Science* (**183**, 853; 1974) that melanins can be made to 'switch' from a poorly conducting to a highly conducting state at fairly low electric fields (say from $10^5 \Omega \text{ cm}$ to $10^2 \Omega \text{ cm}$ at a field of $3 \times 10^2 \text{ V cm}^{-1}$). This remarkable phenomenon occurs both in melanin made synthetically from tyrosine and in that extracted from a human melanoma. The large conduction is not destructive in any way and is reversible; according to some tests, conduction seems to be electronic rather than ionic. Also tests of a few other likely biological materials in the same form (a compressed solid pellet inside a quartz tube, mildly hydrated and of length ranging from 0.1 to 10 mm) suggest that the effect is confined to the melanins and the authors note a similarity in the I - V characteristics of the sample to those of some amorphous inorganic semiconductors which undergo 'threshold switching'. But apart from the major difference in the electric field at which the threshold effect occurs (of the order of 10^3 V cm^{-1} for melanin and 10^5 V cm^{-1} for chalcogenide glasses), the current theory of the inorganic switching phenomenon rests on filamentary conduction, leading to a controlled degree of segregation of the constituents of the glass (for example, segregation of pure tellurium from Ge-Te alloys) and possibly strong injection at the electrodes under the high local fields (Bosell and Thomas, *Phil. Mag.*, **27**, 665-81; 1973).

Neither of these effects seems even likely in the system described, especially since the switching becomes unstable at thicknesses of chalcogenide greater than a few micrometres. Thus, the suggestion of McGinness *et al.* that melanin in the human body can be a cause rather than a by-product of disease and that its mode of action can be related to this 'electronic device' action is probably premature, especially considering the preliminary nature of the experiments. A revival of discussion on *in vivo* electronic effects in some biological materials associated with oxidation-reduction is, however, welcome if only because science has perhaps moved far enough since the 1950s that it can now devise adequate tests for the basic

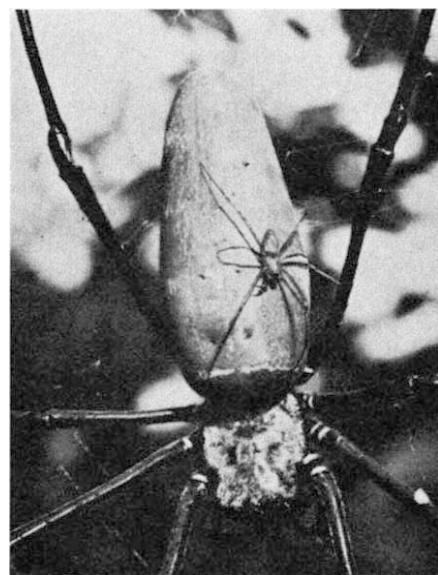
theories of transport in wet solids. Also a new approach to the treatment of melanotic diseases may well be stimulated by this particular revival of an intellectually stimulating discussion.

How to wrap up and get your female

from our
Animal Behaviour Correspondent

MICHAEL and Barbara Robinson spent a year in New Guinea observing the giant wood spider, *Nephila maculata* (Fabricius). Their report (*Smithson. Contrib. Zool.* No. 149; 1973) contains some remarkable facts of spider life, including a description of the strange method of courtship. As can be seen from the photograph, the male is much smaller than the female (4-6 mm body length as opposed to 40-50 mm) and before actual copulation, he goes through an elaborate 'lashing' of the female, weaving silk onto her body. The male may spend the greater part of two days standing on the female and during this time periodically indulges in intense bursts of activity, laying down silk threads between the female's legs and body. The female rarely gives any response at all to this silk deposition, after which the male copulates.

It would seem logical to suppose that the functional significance of binding up the female in this way would be that it in some way restrains her and prevents her from attacking the male. But although a great deal of silk is laid down around the leg bases, this does not seem to hamper the female very much, for she can rush off on predatory excursions even after the silk



Male on dorsal surface of the abdomen of a female *Nephila maculata*. Silk lines are visible on the thorax of the female leading to the leg bases and abdomen.

has been heavily deposited. The Robinsons suggest that the silk may in fact prevent the female from bending at the waist, which would in turn prevent her from picking off the male while he is copulating.

As well as describing the unique courtship, the Robinsons also describe the kleptoparasites of *Nephila*, spiders of other species which live in the web and steal food caught by the host. Often, the kleptoparasites were seen to rush in and start feeding while *Nephila* was actually subduing or wrapping its prey, a somewhat hazardous past-time as the kleptoparasites themselves often became enmeshed.

Origin of antibody diversity

from a Correspondent

THE apparently inexhaustible repertoire of antibody-combining sites possessed by vertebrate animals raises an important question. Does the germ line genome code for all antibody genes (germ line theory), or is diversity generated during the life of an individual by mutation of a restricted number of germ line genes (somatic theory)? Argument surrounding this question has raged backwards and forwards ever since the discovery that heavy and light chains of immunoglobulin molecules are composed of constant (C) and variable (V) regions (Hiltschmann and Craig, *Proc. natn. Acad. Sci. U.S.A.*, **53**, 1403; 1965). What is agreed is that separate genes code for C and V regions and that there is only a single C gene for a given constant region (Ig class or subclass) sequence. The question may therefore be reframed in terms of V genes: are all the conceivable V genes carried in the genome or are they somatically generated?

Perhaps the strongest argument against the germ line theory is the existence of allelic markers and species-specific amino acid sequences in V regions. But this argument is inferential and it has long been the hope that direct experimental evidence could be brought to bear on the question. This now seems to be provided by Premkumar, Shoyab and Williamson (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 99; 1974). In principle, the authors have isolated heavy chain messenger RNA and have estimated by hybridisation the number of C and V genes in isolated DNA. Their results, they argue, firmly support the germ line theory.

An absolute prerequisite for this type of experiment is that the isolated messenger RNA is pure and labelled at high specific activity so that hybridisation can be done in great excess of DNA. In order to achieve this, cells

from mouse myeloma MOPC 315 were labelled *in vitro* with radioactive uridine and cytidine, and then mRNA for the α chain was isolated from the poly(A)-containing fraction taking advantage of its specific interaction with immunoglobulin (Stevens and Williamson, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1127; 1973; *J. molec. Biol.*, **78**, 517; 1973). The resulting RNA sedimented largely as a single peak at 16-17S and, after sonication to 6-7S, was hybridised with about a 10^7 excess of cold DNA, also sonicated to 6.4S.

Using whole mouse embryo (the actual age is not given) as a source of DNA, a biphasic C_{ot} curve resulted. Approximately 70% of the input RNA was hybridised at the maximum, and 20% was found in the low C_{ot} transition. It is here that the authors make a crucial assumption, namely that the low C_{ot} transition ($C_{ot} \frac{1}{2}$ of 1.5) corresponds to hybridisation of V genes and the high C_{ot} transition ($C_{ot} \frac{1}{2}$ of 10^3) represents hybridisation to C genes. If this assumption is wrong, then so are the conclusions. Their reasons for making this assumption are that the RNA is pure, and that the ratio of hybridisation found in the two regions of the C_{ot} curve (0.29) is close to the expected figure of 0.25. The possible occurrence in the isolated RNA of RNA sequence stretches which do not code for C or V region sequences is obviously a source of uncertainty.

Similar, but not quite as impressive, results were obtained when BALB/C spleen or myeloma MOPC 315 DNA was used for hybridisation. Given the basic assumption made, the $C_{ot} \frac{1}{2}$ values correspond to 4-8 C genes and about 5,000 V genes.

Although sequence analysis indicates considerable cross-homology of mouse V_H regions, the figure of 5,000 V_H genes if accepted, must be a lower estimate. If, as the authors assume, that there are an equal number of V_L genes and that V_L and V_H regions can associate at random, then there is sufficient information for at least 2.5×10^7 antibody-combining sites. Even allowing for inefficiency, this would seem to be a high enough figure to account for antibody diversity. The authors conclude that their data "obviates the necessity to search for somatic generators of diversity. Questions concerning the maintenance and control of a large V-gene pool in the germ line and the expression of V genes by interaction with C genes can now be brought more clearly into focus".

Until the fraction of RNA hybridising at the low $C_{ot} \frac{1}{2}$ transition is actually identified as specifying V region, however, proponents of the somatic theory are unlikely to be satisfied and the argument will continue with unabated force.

Sense of supercoils in closed circular DNA

from a Correspondent

AN interesting study on the sedimentation of denatured closed circular DNAs with different degrees of strand interwinding has been made by Schmir, Révet and Vinograd (*J. molec. Biol.*, **82**, 34-35; 1974). From their results these authors have deduced the absolute sense of the supercoils in closed circular DNA and are able to confirm that the binding of intercalating dyes unwinds, rather than winds up, the DNA helix.

Double stranded DNA in the form of closed circular superhelices was first discovered in extracts from polyoma virus in 1963, and has subsequently been shown to exist in a wide variety of organisms. The biological significance of the closure is not yet clear but these molecules have a number of interesting structural and chemical properties. The superhelical structure imposes upon the DNA molecule a topological restraint usually expressed by saying that, for a given molecule, the total number of rotations of one strand about the other, the topological winding number, is invariant. Thus a modification in the number of turns in the basic double helix, for example by changing the ionic strength or pH of the environment, or by the addition of intercalating dyes, is accompanied by an equal and opposite change in the number of superhelical turns. The introduction of a single scission into one of the DNA strands results in a circular DNA with no superhelical structure. The superhelical DNAs sediment at a higher rate than the nicked DNAs, the exact value of the sedimentation coefficient, s , being a complex function of the superhelix density, σ (the number of superhelical turns per ten base pairs).

Vinograd, Lebowitz, Radloff, Watson and Laipis (*Proc. natn. Acad. Sci. U.S.A.*, **53**, 1104; 1965) studied the change in s for a DNA in which the topological winding density, $A \approx 1$, as the pH of the environment is progressively increased and the DNA becomes more denatured. At pH ≈ 12.5 , the DNA duplex is completely denatured, extensive positive supercoiling occurs and the sedimentation coefficient in these conditions is 2.1 times greater than that of a single stranded, circular molecule having an equivalent molecular weight. This greatly increased sedimentation coefficient can therefore be attributed directly to the winding of one strand about the other.

In the new article, Schmir *et al.* extend these results by studying the sedimentation coefficients, at high pH values, of DNAs with different topological winding densities. A number of closed circular DNAs with A values

varying from 0.99 to 0.89 were prepared from simian virus (SV40) and phage PM2 DNAs. This 10% decrease in topological winding density results in a decrease of approximately 5% in the alkaline sedimentation coefficient, which can be understood in terms of a small negative change in the superhelix density of the denatured molecule. The data connect smoothly with similar results obtained by Sebring, Kelly, Thoren and Salzman (*J. Virol.*, **8**, 478; 1971) for DNAs with A values from 0.6 to 0.15. The range is completed by the inclusion of a point for $A \approx 0$ computed from a relation proposed by Wang (*Biopolymers*, **9**, 489; 1970). As A decreases from 1 to 0, the value of relative s changes from 1 to 0.45.

The A values quoted here were determined on the assumption that for all naturally occurring closed circular DNAs, $A \leq 1$. This arises from the interpretation of the experimental results obtained from studies on the binding of intercalating dyes to superhelical DNA in accordance with the proposal by Lerman (*J. cell. comp. Physiol.*, **64**, suppl. 1, 1; 1964) and Fuller and Waring (*Ber. Bunsenges. phys. Chem.*, **86**, 805; 1964) that intercalating dyes unwind the DNA helix. The observed data are then consistent with a model in which the superhelix density σ is initially less than 0 and A is not greater than 1.

The recent assertion by Paoletti and LePecq (*J. molec. Biol.*, **59**, 43; 1971) that ethidium bromide winds up the DNA helix has the corollary that $A \geq 1$ in naturally occurring closed circular DNAs. Schmir *et al.* are able to refute this suggestion completely. First, they point out that the data of Sebring *et al.* were derived from replicating intermediates of SV40 DNA which were extensively unwound while nicked during the replicating process. There can therefore be no doubt that for these DNAs, $A \ll 1$. In addition, the point computed by Wang is unaffected by considerations of winding or unwinding the helix. Second, they have carried out dye titrations with a closed circular replicative mitochondrial DNA before and after removal of a short progeny strand (Révet, Schmir and Vinograd, *Nature new biol.*, **229**, 10; 1971). These experiments indicated quite clearly that less ethidium bromide was required to relax the supercoiled DNA molecule in the presence of the progeny strand than was required after its removal. The removal was achieved in such a way that the parent DNA did not become denatured and was able to rewind into its original structure. The results of Schmir *et al.* indicate unequivocally that the insertion of a displacing progeny strand and the binding of ethidium bromide act in the same direction, that is, they both unwind the DNA helix.

Schmir *et al.* conclude therefore that the initial superhelix density must be negative, A is less than 1 and the sense of the supercoils in natural closed circular DNA is negative, not positive as proposed by Paoletti and LePecq.

The results obtained during the past ten years on the binding of certain dyes to superhelical DNAs can be interpreted almost without exception in terms of a model in which the bound dye produces an unwinding in the DNA double helix. In view of the extreme sensitivity of superhelical DNA to small changes in its structure, this technique might in the future provide a means of distinguishing between the different modes of interaction of DNA and a variety of dye molecules.

Torpor in an Andean hummingbird

from our Animal Ecology Correspondent

THE attraction of hummingbirds to physiologically-inclined ecologists is easy to understand. How do these tiny, active creatures manage to survive in the inhospitable habitats of some of the world's highest mountains? Calder and Booser (*Science*, **180**, 751; 1973) found that nocturnal torpor during incubation was not uncommon at times of energy depletion. When the ambient temperature was low and heavy rain precluded nocturnal foraging, a gradual lowering of the body temperature ensured the sufficiency of the bird's energy reserves to see it through the night.

Another physiological adaptation of obvious survival value has been reported by Carpenter (*Science*, **183**, 545; 1974) who worked with a population of the hillstar hummingbird *Oreotrochilus estella*, which lives at a height of between 3,800 m and 4,300 m in the high Andes. By day the birds forage for nectar from a variety of local and exotic plants. At night they roost communally in caves and can readily be observed with little disturbance. Carpenter recorded body temperatures from a small group of birds: most were recorded between one and eight times during either the summer or winter study periods; a few were recorded in both summer and winter. Torpor was defined as a significant drop from the normal body temperature of about 30° C. In summer the average duration of nocturnal torpor was 7 h and during the winter 10 h. This difference is significant in spite of the fact that winter nights were 15 to 90 min longer than summer nights. Calder and Booser reported that body temperature during nesting torpor never sank below 6.5° C. The same was true in Carpenter's study, even though ambient temperature sometimes dropped to 3° C.

Why is torpor used more extensively



Zoological Society of London

Stripe breasted starthroat hummingbird (*Heliomaster squamosus*), a different species of hummingbird from that described by Carpenter but it shows the characteristic feeding behaviour of these birds.

by the birds in winter? One of the winter groups of birds lives in an area surrounded by blooming *Eucalyptus* trees, from which they fed heavily. Incidence and duration of torpor was the same as in other local populations with less well endowed larders. It seems as if energy depletion is not a cue for torpor. Nor does temperature seem to be the trigger. In summer the ambient temperatures in the roosts varied from 3.5° C to 13° C, and in winter from 3.0° C to 13° C. This high winter temperature is thought to be exceptional; winter roost temperatures are normally near freezing.

Carpenter suggests that although the difference between summer and winter ray length is brief, it is sufficient to act as a photoperiodic cue for a circannian rhythm. Such a rhythm would have survival value for a species living in an area where nocturnal temperature is seasonally low and in which the day time temperature gives no indication of the ensuing night time level. By entering torpor as soon as it roosts, a bird is capable of regulating its body temperature for long periods should the ambient temperature drop much below 6.5° C—a clever way of extending fuel contingency reserves without increasing fuel capacity.

Probing the secrets of ionic channels

from a Correspondent

SINCE Galvani's fortuitous joining of brass hook, iron plate and frog in 1786, the lure and mystery of 'animal electricity' have been reduced to the molecular secrets of so-called cation channels, which intermittently allow these ions to cross membranes. Recent chemical and electrical probes of the structure of these channels were the main topics of a meeting at the Royal Society on March 13-14.

The simplest model of a channel is a pore, a few Angstroms in diameter, capped by one or more gates which open or close the pore depending on the recent history of the voltage across

the membrane. B. Hille (University of Washington) summarised the evidence for such models for the sodium and potassium channels, as gleaned from the voltage clamp technique pioneered by Hodgkin and Huxley more than 20 years ago. Hille's and other data (Woodhull, *J. gen. Physiol.*, **61**, 687; 1973) suggest the presence of a carboxylic acid group inside the sodium pore.

The same carboxylic acid group was detected by J. M. Ritchie (Yale University) and colleagues, using a powerful technique not requiring electrical measurements (*J. Physiol.*, **232**, 53P; 1973). They deduced its presence from the competitive binding of tritiated tetrodotoxin (TTX) and other ions to the sodium channel. TTX binding survives solubilisation of membranes in Triton X-100, but the sodium channels have yet to be purified by this route. By contrast, the much tighter binding of α -bungarotoxin to acetylcholine receptors has aided their purification, as discussed by D. P. Green (University College, London).

The lack of a specific toxin for the potassium channel was widely lamented, though vagaries of the potassium currents in nodes of Ranvier and in cardiac muscle were described by B. Frankenhaeuser (Karolinska Institute, Stockholm) and D. Noble (University of Oxford). P. F. Baker (University of Cambridge) discussed delayed movements of calcium following action potentials, which he thinks are related to those activating transmitter release at synapses. L. G. M. Gordon and D. A. Haydon (University of Cambridge) talked about quantal permeability changes in artificial membranes, and G. K. Radda (University of Oxford) described the use of fluorescent probes.

A. von Muralt (Universität Bern) and J. V. Howarth (Marine Biological Association, Plymouth) summarised the slight optical and thermal changes in membranes during action potentials. These, it was concluded, reflect the consequences rather than the causes of the underlying permeability changes.

The latest and most controversial technique involves measuring the tiny displacement currents visible in voltage-clamped axons when the usual ionic currents are blocked. C. M. Armstrong and F. Bezanilla (University of Rochester) think that these are 'gating currents', caused by the movement of those charged particules or dipoles thought to open and close the sodium channel (*Nature*, **242**, 459; 1973), and they reported, as supporting evidence, that perfusion of an axon with ZnCl_2 removed reversibly both sodium and displacement currents. Both currents could also be inactivated by depolarising pulses, and this effect was removed by pronase.

H. Meves (MBA, Plymouth) took

issue with this interpretation, pointing out that other charged groups, which may vastly outnumber the gating groups, could easily obliterate the true gating current. Furthermore, although the qualitative behaviour of the measured displacement currents is acceptable, he observed that they depart from expectations in several quantitative respects. W. K. Chandler (Yale University), who first studied similar but slower currents in muscle (*Nature*, **242**, 244; 1973), suggested that they play a part in excitation-contraction coupling.

If gating currents are what their name implies, then their magnitudes may be used to calculate the density of sodium channels, if one assumes six net charges per channel as given by Hodgkin and Huxley. R. D. Keynes (University of Cambridge), who organised the conference, and E. Rojas (MBA, Plymouth) estimated on the basis of both displacement currents (*J. Physiol.*, **233**, 28P; 1973), and TTX binding, that the squid giant axon has about 400 sodium channel per μm^2 . A. L. Hodgkin (University of Cambridge), using a dimensional argument, suggested that the density of sodium channels in nerve should be about 1,000 per μm^2 , if the conduction velocity is to be maximised. These numbers are much higher than the 13 channels per μm^2 originally estimated on the basis of TTX binding to lobster nerve (Moore, Narahashi and Shaw, *J. Physiol.*, **188**, 99; 1967) and popularly subscribed to until about the middle of this meeting. The discrepancy suggests either vast species differences, or errors in the interpretation of TTX binding studies (tritiated impurities were mooted), or that 'gating' currents contain a large component that has nothing to do with gating. *Caveat emptor.*

Cloning for obesity

from our
Molecular Genetics Correspondent

NATURAL selection of organisms for traits desirable under particular circumstances is of course a well established feature of evolution. And artificial selection has been used both to isolate mutants of bacteria or eukaryotic cells in culture and to permit only cells possessing certain functions to survive. Cloning cells in culture can be used to isolate lines with enhanced or reduced expression of functions which can be followed quantitatively (rather than the all or nothing expression of many mutants). In a classic series of experiments some years ago, the 3T3 and 3T6 cell lines of mouse fibroblasts were derived with different responses to inhibition of growth by culture conditions. By cloning sublines of the 3T3 cell, Green and Kehinde now report in

the March issue of *Cell* (**1**, 113; 1974) that they have isolated two sublines which accumulate large amounts of triglyceride fat in the resting state.

Illustrating their article with some striking colour photographs, Green and Kehinde demonstrate vividly that the cells of these sublines resemble adipose cells in accumulating large amounts of lipid in their cytoplasm. The initial observation which led to these experiments was the presence of a few cells possessing liquid droplets in resting cultures of 3T3 cells; when resting cells are transferred to conditions which promote growth, the fatty cells are not found among the growing progeny, although such cells reappear when the culture again enters the resting state. By isolating areas rich in lipids from resting cultures, growing these isolates and then repeating the process when the culture reached the next resting phase, Green and Kehinde obtained cultures which were progressively richer in fatty cells. Eventually, they isolated two clones which accumulate lipid in large amounts.

When resting cultures of these cells are transferred to conditions which stimulate growth, the most fatty cells may survive for a few days but usually disintegrate eventually. The presence of large amounts of fat presumably interferes with the processes of cell division. But cells possessing less fat may reinitiate division; and after a successful division — sometimes mitosis fails in cytokinesis — the amount of lipid is diluted and then continues to decrease in each cell during successive cycles; except for the extremely fatty cells, therefore, both the lipid-accumulating sublines can be propagated indefinitely.

What is the cause of the lipid accumulation? Possible explanations, at present unresolved, include excessive uptake from the culture medium, increased synthesis within the cell or decreased degradation. Because the accumulation of lipid is inhibited by lipolytic agents such as dibutyryl cyclic AMP, Green and Kehinde suggest that one practical use of these lines may be to provide a means to test drugs for possible effects on lipid metabolism. And even more intriguing are some of the possible theoretical implications of the properties of these cells. What genetic change may underlie the conversion of a cell derived from the fibroblast into one apparently akin to the cells of adipose tissue? How is this change related to the usual processes of differentiation in developing mouse cells and can such changes be found for other phenotypes? The development of a differentiated phenotype in culture represents an important contrast with the more usual consequence of tissue culture.

Spatial resolution of ERTS images by computer

Alden McLellan & William Kuhlrow

Space Science and Engineering Center, Institute for Environmental Studies, University of Wisconsin, Madison, Wisconsin

The authors discuss a solution to one of the problems concerned with the use of the large amounts of data now available from remote sensing satellites.

A PERSISTENT problem in the remote sensing of Earth resources is the difficulty of gaining efficient access to the vast quantities of data which have been generated. Second only to this problem is that of displaying the results in a form convenient for analysis. Ever since the advent of remote sensing satellites, one of the aims of imagery analysis has been to modify the data in order to delineate and enhance specific features within a composite display. The aim of many investigators of data handling is to develop a man-computer integrated system for quickly and accurately enhancing and interpreting a large volume of data from an Earth resources satellite. Since the early 1960s, there has been extensive research on the development of spectral signature and pattern recognition theory in processing multispectral data. The thrust of this research has been to develop computer software packages to analyse large amounts of data generated by multispectral scanning instruments. It has been only recently, however, that image-display man-computer hardware systems have been developed.

Feasibility

The University of Wisconsin's Space Science and Engineering Center (SSEC) has demonstrated the feasibility of the Man-computer Interactive Data Access System (McIDAS) which has rapid data storage, access, display and analysis techniques applicable to many areas of satellite data processing. In this field, it is of considerable value to reference small subsets of these data in a fast and economical way, with accompanying observation and guidance by a human operator who interacts with the system. The advantage of the McIDAS system in the analysis of ERTS data is that the operator can see the data displayed on a television screen in a form suitable for easy interpretation by varying such quantities as the brightness and colour enhancement functions, time lapse motion and scale sizes. He can make the appropriate judgments, then communicate exact coordinates and instructions for processing of the digital data. In fact, the system always has access to the full precision data, so that maximum accuracy is always possible.

We are developing the software programs specifically for this system to analyse remote sensing data from satellites for natural resource inventory purposes and for monitoring changes in land use. Normally, investigators of Earth resources use aerial photographs to obtain inventory estimates of the required accuracy but not without expending time in the preparation of photographs, tedious interpretation of them and data handling. Lins and Milazzo¹ have described the use of multispectral photography for detecting land-use change. Wray² has made a comparative study of metropolitan regions using ERTS imagery; he states that the imagery shows great promise for the inventory of gross land use and the monitoring of changes in land use³.

The purpose of this paper is to present results from McIDAS showing the spatial resolution that can be obtained from ERTS-1 digital data by the display of urban images appropriate for identification of land use.

Four reels of ERTS-1 system corrected computer compatible tapes from the multispectral scanner (MSS), containing a scene of eastern Wisconsin taken on September 14, 1972, was made available to us by NASA. After modifying the data format for the McIDAS system, the Milwaukee metropolitan area was displayed in all four channels sequentially. A number of images were then manually enhanced by means of a teletype keyboard in which various levels of brightness values of the raw ERTS data were transformed to a chosen level of brightness within the dynamic range of the Muirhead PhotoFax Receiver. In the case of the pictures shown here, this involved 128 brightness levels from the MSS 5 channel transformed nonlinearly into 256 levels for the Muirhead Receiver. By using a number of trial and error non-linear enhancement curves to display the images on the McIDAS CRT, we were able quickly and economically to find the enhancement curve suited to the display of urban imagery in such a way that a number of recognisable features can be seen.

In Fig. 1, the major highways and roads leading to Milwaukee can be easily identified. The downtown area is located near the centre of the picture where East-West Interstate 94 turns South. An effluent plume into Lake Michigan can be seen immediately to the south-east of the downtown area just beyond the place where the Menomonee and Milwaukee Rivers merge. Other land features can also be seen, such as the Milwaukee County Stadium and the Forest Home Cemetery.

The area shown in Fig. 2 is one-fourth that encompassed by Fig. 1. In Fig. 2, the individual data points which make up the imagery become somewhat discernible. The plume at the estuary of the rivers, as well as the variation in reflected radiance from Lake Michigan near the shoreline east of Mitchell Field, is more apparent than in Fig. 1.

In Fig. 3, the individual ERTS data points are now quite evident. Mitchell Field appears here as a pattern of blocks.



FIG. 1 ERTS-1 Mufax picture from McIDAS digital computer enhancement of Milwaukee, Wisconsin. MSS 5 red visible channel taken on September 14, 1972. The picture comprises 360 ERTS scan lines (27.4 km) and 540 ERTS elements (30.8 km). a, Milwaukee river; b, Lake Michigan; c, Menomonee river; d, stadium; e, plume; f, highway 94; g, cemetery; h, Mitchell Field.



FIG. 2 ERTS Mufax picture from McIDAS digital computer enhancement of central and south Milwaukee. MSS 5 red visible channel taken on September 14, 1972. The picture comprises 180 ERTS scan lines (13.7 km) and 270 ERTS elements (15.4 km). *a*, Pier; *b*, plume; *c*, highway 94; *d*, Lake Michigan; *e*, cemetery; *f*, golf course; *g*, Mitchell Field.



FIG. 3 ERTS-1 Mufax picture from McIDAS digital computer enhancement of South Milwaukee. MSS 5 red visible channel taken on September 14, 1972. The picture comprises 90 ERTS scan lines (6.8 km) and 135 ERTS elements (7.7 km). *a*, Lake Michigan; *b*, golf course; *c*, Mitchell Field.

The limits to the ability of ERTS to resolve features is clear; any further enlargements would not improve spatial resolution for the viewer. Other enhancement curves would, however, help delineate particular features not considered here.

Our software programs developed so far allow for three sizes of image field which represent the magnification ratios of 1:2:4, designated by the last digit on the annotation block in the three figures as 3, 2 and 1 respectively. The following information concerns Fig. 3. Information corresponding to Figs 1 and 2 can be obtained simply by using the scale factors 4 and 2, respectively.

In Fig. 3 the total image field represents 90 ERTS scan lines with 135 ERTS samples comprising each line. The 90 lines $\times$ 135 samples represents a physical field size of approximately 6.8 km $\times$ 7.7 km, respectively. Because of the size of the individual data points represented in the scanning display, each ERTS element had to be repeated twice to ensure that the proportions of the imaged field would correspond closely to the actual geographic proportions. The scaling, however, is still not quite exact. In the figures, the vertical dimension of the image is 'stretched' by 6.8% with respect to

the horizontal dimension. The enhancement curve for each of the figures shown was:

Raw	$\left\{ \begin{array}{l} 0 \text{ to } 7 \rightarrow 0 \\ 8 \text{ to } 64 \rightarrow 0 \text{ to } 255 \text{ (linear)} \\ 65 \text{ to } 128 \rightarrow 255 \end{array} \right\}$	Enhanced display
ERTS		
digital data		

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Mesozoic thermal events in southeast China

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The first Rb-Sr age data are reported for Mesozoic batholiths in southeast China. Two thermal episodes are recognised (165 ± 13 and 90–109 Myr) and can be correlated with the periods of rapid spreading of the Mesozoic Pacific Ocean floor as proposed by Larsen and Pitman.

FROM the study of Mesozoic magnetic lineations in the western Pacific, Larsen and Pitman¹ and Larsen and Chase² postulate that, during the late Mesozoic, the Pacific sea

floor was spreading from at least five spreading centres joined at two triple points. Most of the Pacific Basin today is occupied by the expanded Pacific plate of the late Mesozoic system. This implies that an area equal to most of the Pacific Basin has been subducted beneath the surrounding continents since the early Cretaceous. Larsen and coworkers also correlate the Keathley magnetic lineations of the North Atlantic with the Hawaiian lineations of the Pacific and suggest that the Bay of Biscay opened during the interval between 150 and 110 Myr BP and the drift in the South Atlantic was initiated at sometime during the interval from 110 to 85 Myr BP. More importantly, with their magnetic

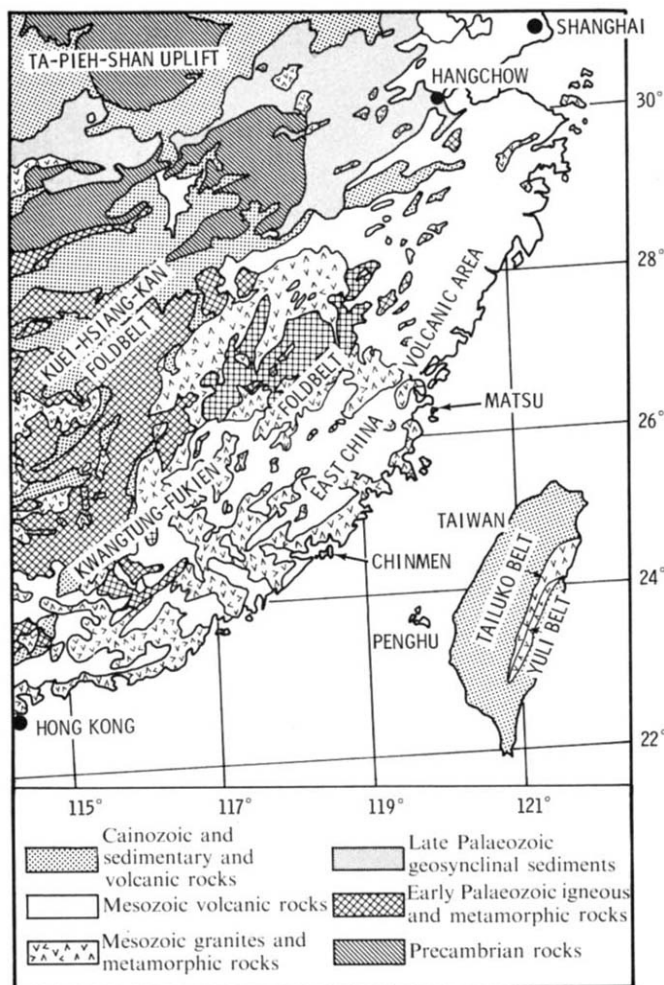


FIG. 1 Generalised geological map of southeastern China and Taiwan. Source, refs 11 and 28.

reversal time scale, they show that from 110 to 85 Myr there was a pulse of very rapid spreading in the central and south Atlantic and throughout the Pacific (up to 18 cm yr⁻¹ along the Phoenix-Pacific spreading centre).

With a rigid plate assumption, rapid spreading corresponds to rapid subduction and consumption of oceanic plate materials beneath the surrounding continents. (This statement is not strictly true, for subduction may be beneath oceanic but not continental lithosphere, for example, in the western Pacific. However, from an analysis of tectonic activities along the Pacific margins during the Mesozoic time, this statement is generally true.) If andesitic or granitic magmas of continental margins and island arcs were derived from the under-thrust lithosphere or had any genetical relationship with the subduction, one may anticipate a correlation between spreading rate and the intensity of orogeny and associated thermal episodes (andesitic volcanism, batholithic intrusion and metamorphism). Indeed, Larsen and Pitman¹ related the pulse of rapid spreading from 110 to 85 Myr BP to episodes of circum-Pacific intrusive and extrusive activity and orogenic movement during this period.

Here, I report a new set of age data on some granitic batholiths of southeast China. I shall show that these new data support the postulate of Larsen and Pitman.

The geotectonic element of which the southeast China coastal region is a part was named 'Cathaysia' by Grabau². It includes the South China Basin, Taiwan, the Yellow Sea and Japan, and it was suggested to be an 'old land' of Precambrian age. This 'old land' supplied detritus to a Cathaysian geosyncline to the northwest, only to disappear as the

geosyncline later migrated southeastward. But Hsieh⁴ rejected Grabau's concept of an 'old land' and suggested that the bulk of the Kuei-Hsiang-Kan and the Kwangtung-Fukien Foldbelts (Fig. 1) are younger Caledonian, and postulated a Hercynian foldbelt north of Canton (not shown in Fig. 1, but located about 50 km northwest of Hong Kong) and a larger Mesozoic foldbelt along the coastal region of southeast China.

The basement rocks of southeast China are principally metamorphic rocks of various facies associated with intrusive granites. Their ages are not known. Toward the coastal areas, volcanic rocks, also associated with intrusive rocks, comprise the widespread East China Volcanic Area. It has been speculated that they are of Jurassic-Cretaceous age. During the Mesozoic Yenshan Orogeny in China (whose early phase corresponding to the Nevadan Orogeny of the western United States) folding and faulting of sedimentary rocks were accompanied by volcanism and intrusion. Extensive Mesozoic granites were thus developed throughout the southeastern part of China. The period of Yenshan Orogeny includes the most important metallogenetic epochs of the whole of China⁵.

Dating the rocks

Rocks collected from two offshore islands (Chinmen and Matsu) were dated by the Rb-Sr method. All granitic rocks from Chinmen and Matsu, including granitic gneisses and biotite-hornblende gneisses, yielded mineral isochron ages of 93 to 109 Myr (Table 1). A late pegmatitic dike (K-4), which intruded a granitic body (K-3), was dated at 90 Myr. The initial ⁸⁷Sr/⁸⁶Sr ratios of these rocks vary with the Rb/Sr ratios of the individual rocks from 0.706 to 0.716. Dioritic and gabbroic rocks from Matsu yield similar ages (94.1 to 97.5 Myr), with a very narrow range of initial ratios of about 0.7065 to 0.70695. In addition, a primary age of 165 Myr, with an initial ⁸⁷Sr/⁸⁶Sr = 0.7055, was obtained from the whole rock data for granitic rocks, implying either that these rocks may be genetically related and of mantle origin with limited crustal contamination or that the original sedimentary sources for the granitic gneisses were Sr isotopically homogenised about 165 Myr ago.

The whole rock data for the mafic rocks from Matsu (diorites, gabbro and spessartite) are more scattered and

TABLE 1 Mineral and whole-rock isochron ages and initial ⁸⁷Sr/⁸⁶Sr ratios of some igneous rocks from Southeastern China

Sample No.	Locality	Rock type	Age (Myr)	(⁸⁷ Sr/ ⁸⁶ Sr) ₀
K-1	Chinmen	granite gneiss	95 ± 2*	0.7084 ± 1*
K-2	"	bio-hb gneiss	96.7	0.7060
K-3†	"	granite gneiss	109 ± 7	0.7079 ± 5
K-4‡	"	pegmatite	90 ± 3	0.7112 ± 1
K-5	"	granite gneiss	94.2 ± 3	0.7159 ± 5
K-6	"	granite gneiss	98.1 ± 12	0.7065 ± 7
K-7	"	bio-hb gneiss	—	0.7066‡
M-23	Matsu	diorite	97.2 ± 1.4	0.70693 ± 1
M-24	"	hb-porphyrityte	—	0.7062‡
M-25	"	gabbro	97.5 ± 4.8	0.70695 ± 8
M-27	"	diorite	94.1 ± 0.3	0.70666 ± 3
M-31	"	spessartite	—	0.7065‡
M-06	"	granite	95.5 ± 4.0	0.70676 ± 8
MPK	"	granite	92.6 ± 2.0	0.70691 ± 3
Whole-rock§	Chinmen & Matsu	granites	165 ± 13	0.7055 ± 2

* Ages were calculated by the York method³⁰ using the decay constant of ⁸⁷Rb = 1.39 × 10⁻¹¹ yr⁻¹. Uncertainties for both ages and initial ⁸⁷Sr/⁸⁶Sr ratios are at 2σ level except for K-2 which is a two-point isochron. Uncertainties of initial ratios correspond to the last figures.

† K-3 was intruded by K-4 pegmatite dike.

‡ Initial ⁸⁷Sr/⁸⁶Sr ratios corrected for radiogenic ⁸⁷Sr growth in the assumed time interval of 95 Myr.

§ Whole-rock isochron was constructed using the data of K-1, K-2, K-3, K-5, K-7, M-06 and MPK.

TABLE 2 Mesozoic thermal events in circum-Pacific areas (based on radiometric dates)

Area*	Description and comment	References
Alaska-Aleutian Range	Three discrete episodes of intrusions are found: (1) early to middle Jurassic (176-154 Myr), (2) late Cretaceous and early Tertiary (83-58 Myr), (3) Middle Tertiary (38-26 Myr). All ages are K-Ar mineral dates.	14
Coast Range, British Columbia	Orogenic pulses took place from Proterozoic to late Tertiary. K-Ar dates suggest that plutonic emplacement and later uplift were most vigorous and widespread in Cretaceous and Tertiary.	15
Sierra Nevada	K-Ar and Rb-Sr data show that intrusion began in late Triassic (210 Myr) and ended in late Cretaceous (80 Myr). Emplacement of granites accomplished in five major epochs, regularly separated by 30 Myr intervals. Magmas were derived from the upper mantle or lower crust with some sialic contamination.	7, 16, 17
Franciscan Formation	K-Ar dates span from 150 to 70 Myr. Three major metamorphic events: (1) 150 Myr, (2) 90-110 Myr, (3) 70 Myr. These events are correlated with intrusive epochs in the batholith belt (example, Sierra Nevada).	9
SW United States	K-Ar dates indicate two broad patterns which culminate in (1) the Jurassic (160-130 Myr) and (2) the mid- to late Cretaceous (105-75 Myr). Essentially coeval with Franciscan and Batholith belt activities.	8
Baja California	U-Pb age = 96-118 Myr. Rb-Sr data indicate a mantle source for the Peninsular Range batholith.	18
Northern Chile	K-Ar biotite ages indicate four episodes of granitic intrusion: (1) lower Jurassic (182-190 Myr); (2) middle Cretaceous (87-107 Myr); (3) lower Palaeocene (60-67 Myr); (4) upper Eocene (40-43 Myr). Intrusive foci migrated eastwards.	19
Southern Chile	K-Ar and Rb-Sr ages indicate three major episodes of intrusion into the basement complex of Palaeozoic age: (1) 164-126 Myr; (2) 106-80 Myr; (3) 53-11 Myr. (Original Rb-Sr ages were recalculated using Rb decay constant = $1.39 \times 10^{-11} \text{ yr}^{-1}$).	20
Antarctica	Granites and granodiorites from the peninsular region of western Antarctica show a pattern of Mesozoic igneous activity similar to that of South Andes region. Rb-Sr ages clustered around 96-109 Myr.	21, 22
New Zealand	Radiometric dates for intrusive and metamorphic rocks on both sides of the Median Tectonic Line of the South Island cluster in the interval 95-120 Myr. The timing of the Cretaceous orogeny and paired metamorphism in New Zealand is similar to that in Japan.	23
Southeast China	Rb-Sr W. R. isochron age of 165 Myr suggests a primary thermal event for the Chinmen granites. Mineral isochron ages, which cluster in the interval 94-109 Myr, correspond to a secondary thermal event for the Chinmen granites and a primary intrusive event for the Matsu diorites and gabbros.	This paper
Taiwan	K-Ar age of 86 Myr for a muscovite from a quartz diorite which intruded agneissic rock in the Tailuko Belt (low P/T), immediately west of the Yuli Belt (high P/T type). The time of intrusion corresponds to that of the Nanao Orogeny in Taiwan and an episode of the Yenshan Orogeny in China.	12
SE Asia	Granites northeast of Gulf of Thailand are mainly Triassic or older. Widespread thermal events including granitic emplacement took place during Cretaceous to Tertiary. In Indonesia, granites from the Lassi Mass and the Lampong Mass of Sumatra and from offshore areas north of Java were dated at about 100 Myr.	24, 29
Japan	K-Ar dates indicate that most intrusion took place in early Cretaceous to early Tertiary (90%), and weakly continued to late Tertiary. Paired metamorphism occurred during middle Cretaceous time.	25, 26
South Korea	Rb-Sr data indicate that a thermal event (180 Myr) homogenised Sr isotopic compositions of mineral phases in a Precambrian rock. Whole rock data of five granitic rocks from the southeast coastal region yield an age of about 100 Myr.	27

* Areas arranged clockwise around the Pacific.

do not form any linear isochron relationship (details to be published by B. J., P. Y. Chen and T. P. Yen). Their initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of 0.7065 to 0.70695, derived from mineral isochrons, suggest a possible mantle origin with some crustal contamination. It is quite certain, however, that the source materials for the Chinmen and Matsu granites were not solely Rb-rich crustal rocks of much older ages, such as the Precambrian strata of the Ta-Pieh-Shan Uplift.

In summary, the granitic rocks probably record a primary (165 Myr) and a secondary (about 100 Myr) thermal episode, while the unmetamorphosed diorites and gabbro from Matsu register only a time of intrusion also at about 100 Myr. The time interval of 90 to 109 Myr embraces an outstanding episode of the Mesozoic Yenshan Orogeny in China.

It is of great interest to see how the rapid seafloor spreading during the time interval of 110-85 Myr BP correlates with supposedly consequential dynamic events on the continents, especially in the circum-Pacific areas. Gilluly⁶ stated "The Middle Cretaceous was the time of emplacement of by far the greatest plutons of Phanerozoic time in the whole

belt extending from Alaska to Baja California. The batholiths of the Peninsular Range, the Sierra Nevada, many of the Coast Range plutons of California, the Idaho Batholith and many of its satellites in eastern Oregon and northern Idaho all belong to this episode. The plutons of northeastern Washington are outliers of the great Coast Range batholith of British Columbia." To supplement the evidence cited by Larsen and Pitman, I have collected some more supporting evidence shown in Table 2. Not all the age data in Table 2 explicitly indicates a strong peak during the 85 to 110 Myr interval in the age distribution pattern. This is easily understood if factors such as sampling bias and rates of uplift and subsequent erosion which exposed the intrusive rocks are considered. Furthermore, even during a period of rapid spreading, the rates of lithosphere consumption and accompanying igneous activities will not necessarily be increased uniformly around the Pacific margins. The possibility of a fortuitous coincidence between the fast spreading indicated by the magnetic anomaly time scale and the magmatic episodes should not be overlooked. Perhaps Larsen and Pitman's time scale is premature and may be changed after

more detailed study. Similar doubts about such a correlation and about the regular periodic magmatic events proposed by Evernden and Kistler⁷ for the Sierra Nevada were raised by Armstrong and Suppe⁸. Nevertheless, the present data for China are consistent with the interpretation of Larsen and Pitman.

Metamorphism with intrusion

Finally, I would like to call attention to the coeval occurrence of paired metamorphism and intrusion on both sides of the Pacific during the Mesozoic. On the east side the Franciscan terrain is parallel to and west of the batholith belt of California. The metamorphism of the Franciscan Formation occurred from 150 to 70 Myr BP, a time span which is essentially contemporary with the times of most extensive magmatism in the batholith belt⁹.

On the west side of the Pacific, there are the classical examples of paired metamorphic belts in Japan¹⁰, and the Yuli Belt of high pressure/low temperature glaucophane schist in Taiwan, about 170 miles east of Chinmen and Matsu (Fig. 1). Since no systematic radiometric dating has been done on these metamorphic rocks, a direct correlation is not possible. Based on field relationships, however, the metamorphism was thought to occur in late Mesozoic¹¹. Moreover, as shown in Table 2 a single K-Ar date of 86 Myr¹² was reported for muscovite from a quartz diorite which intruded a gneissic rock of the Tailuko Belt, with which the Yuli Belt is in fault contact. Thus the high-pressure type of metamorphism in Taiwan may be coeval and tectonically paired with the low-pressure type of metamorphism and plutonic intrusion in southeast China. This mirrors the situation in California. Yen¹¹ proposed that the Tailuko (low P/T type) and the Yuli belts (high P/T type) in eastern Taiwan (Fig. 1) formed the paired metamorphic belts which are now in direct fault contact. If the so-called arc-trench gap¹³ was ever present between these two metamorphic belts, it would require a great amount of crustal displacement to bring these two together. Perhaps a more logical way is to pair the Yuli belt with the batholiths of southeastern China. This specific problem will be discussed elsewhere (B. J., P. Y. Chen, and T. P. Yen, to be published).

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Free precession of neutron stars

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Wobble in pulsars can provide a clue to the structure and properties of the associated neutron stars.

DETECTION of free precession represents one of the most promising methods of determining both the mass and internal structure of a neutron star. The free precession frequency differs by some three (or more) orders of magnitude, depending on whether the star possesses a solid or a liquid core¹. Thus if one attributes the 35 d period of the high-low X-ray cycle observed for the compact X-ray source Her X-1 (ref. 2) to free precession of the neutron star Her X-1 (refs 3, 4) then from the magnitude of that period alone one can conclude that Her X-1 possesses a solid inner core

(of hyperons or neutrons)⁴. Here we examine ways of exciting and maintaining such precession, as well as mechanisms which act to damp it. This problem has been considered earlier by Goldreich⁵ and by Henriksen *et al.*⁶, whose starting assumptions and conclusions differ somewhat from ours. We conclude that crust-liquid coupling is probably the principal source of wobble damping and that for pulsars wobble excitation is more easily accomplished in a comparatively young, rapidly rotating neutron star. For the Crab and Vela pulsars we find that wobble damping is comparatively ineffective, so that a moderately effective pumping mechanism suffices to excite stellar wobble to an amplitude ($\sim 6^\circ$ to 15°) limited only by the maximum storage of angular elastic energy in the solid crust (or core) of the star.

Analogy with terrestrial wobble

To put the problem of exciting stellar wobble in a terrestrial perspective, we note first that 80 years after the discovery of the free precession of the Earth by Chandler in 1891, the mechanism which is responsible for exciting this Chandler wobble remains in doubt. It is now generally believed that the dissipation in shallow oceans of energy associated with polar tidal motions is sufficiently great that, left to its own devices, the Chandler wobble would damp in less than 100 yr (ref. 7). Yet after almost 100 yr of observation, the amplitude of the wobble (~ 6 m or 10^{-6} rad) remains essentially unchanged; a mechanism to pump the Chandler wobble is clearly required.

Three of the various suggested terrestrial pumping mechanisms are of particular interest to the astrophysicist interested in pumping the wobble of a neutron star: (1) random pumping by earthquakes^{8,9}; (2) random pumping by the core-mantle interaction¹⁰; (3) resonant pumping by earthquakes as a result of in-phase release of angular elastic energy, triggered by the Chandler wobble itself¹¹.

The first mechanism is too inefficient to maintain the Chandler wobble at its present amplitude¹²; its analogue may, however, be operative for a comparatively young neutron star with a solid core (the Vela pulsar?). To the extent that the interpretation of the giant Vela spin ups as produced by corequakes¹³ is correct, such corequakes could excite a substantial stellar wobble through the discontinuous jumps in the centre of nutation they bring about.

An analogue of the second mechanism—random angular momentum transfers between the core and crust of a neutron star—could well act to maintain the precession of a neutron star with a liquid interior (the Crab pulsar?). We lack, however, any physical picture for what might be causing such transfers (which would be reflected as frequency jumps analogous to the changes in the length of the day observed for the Earth); moreover, because of its randomness, such a mechanism is comparatively inefficient, so that pumping a large amplitude stellar wobble in this fashion may be difficult.

An analogue of the resonant pumping mechanism might operate in a neutron star like Her X-1, for which it has been suggested⁴ that the free precessional motion of the star acts to regulate the accretion of matter to the stellar surface. If corequakes in the solid core of Her X-1 are triggered by such accretion (and crustquakes could provide the catalyst needed to bring this about), we would then have a quite efficient self-regulating system: on the one hand, stellar wobble regulates the accretion of matter to the stellar surface (which we see on Earth as a high X-ray state); on the other hand, corequakes triggered by that accretion act to maintain the core wobble. Although the direct detection of such corequakes would be difficult (even in the case of a frequency jump, $\Delta\Omega/\Omega$, as large as 10^{-6}) such quakes could be indirectly detected through the changes in amplitudes of the stellar wobble they produce and may indeed have already been detected. A sizeable jump in the amplitude of the stellar wobble would alter the pattern of the high-low X-ray states, so that one would observe the high X-ray state for a greater (or smaller) number of days within the ~ 35 d overall cycle. Since such shifts in the high-low X-ray cycle have been frequently seen, it is tempting to attribute them to shifts in the amplitude of the stellar wobble; more work is, however, needed before such an interpretation can be taken seriously.

Pulsar mechanism

In a pulsar there is an additional mechanism which might be responsible for exciting stellar wobble—the radiation torque associated with the pulsar magnetic field. Goldreich⁵

has examined the change in nutation amplitude brought about by the electromagnetic torque associated with magnetic dipole radiation; he finds that free nutation grows or damps on the slowing down time scale ($T \sim 2,500$ yr for the Crab pulsar) depending on whether the angle, χ , between the magnetic axis and elastic reference axis exceeds or is less than $\sim 55^\circ$; the rate of growth is, however, proportional to the amplitude to nutation, so that it seems difficult to achieve an appreciable nutation amplitude in times which are not long compared to T . We have examined the problem from a somewhat different point of view; in considering the influence of a torque which acts perpendicular to the reference axis, $\mathbf{n}_0$, we found that the effect of such a torque depends on whether it is fixed in the star or adjusts to the reference plane formed by the angular momentum $\mathbf{L}$ and the reference axis, $\mathbf{n}_0$ (ref. 14). In the former case, the maximum angular amplitude of the stellar wobble is $\sim 1/\Omega\omega\tau_1$, where (L/τ_1) is the magnitude of the external torque; in the latter case, the wobble amplitude increases linearly with time, with a growth rate independent of its amplitude; the maximum amplitude is then determined by either the elastic properties of the stellar crust or by crust-core coupling which may act to damp the wobble motion. We found that in order to achieve nutation amplitudes sufficiently large to be observable (either directly or through microquakes induced by the corresponding growth in the angular part of the elastic energy stored in the crust), we require a torque fixed in the reference plane, L/τ_1 , which is large compared to the slowing down torque, L/T .

A possible candidate for such a torque is a component of the stellar field, $\mathbf{B}_0$, which is sensitive to Ω and the actual figure axis; such a field, instead of being fixed in the star, adjusts to the reference plane and gives rise to an effective torque in the star (as viewed by a corotating observer)

$$\mathbf{N}_{\text{eff}} = -\epsilon_B I_0 (\Omega \times \hat{\mathbf{B}}_0) (\Omega \cdot \hat{\mathbf{B}}_0) \quad (1)$$

where ϵ_B is the 'magnetic' stellar oblateness

$$\epsilon_B = \frac{\alpha B_0^2 R^3}{4(A+B)} \quad (2)$$

and $\hat{\mathbf{B}}_0$ is a unit vector in the direction B_0 . In equation (2) α is of order unity, whereas A and B measure the gravitational and elastic energy stored in the star. (For a self-gravitating incompressible sphere, $A \simeq (3/25) GM^2/R$, and $B = (57/50) \mu V_c$ for a star of radius R with solid crustal (or core) material of volume V_c and shear modulus μ .) The torque (1) is independent of the wobble amplitude for small amplitudes; it is of order of magnitude $10^{39} \sin 2\chi$ for typical stellar fields and can, in principle, be several orders of magnitude larger than the slowing down torque; it would, however, be observable only through its influence on the wobble amplitude, which would, under these circumstances, be sufficiently large both to bring about microquakes in the star and to be directly detectable. The question then reduces to the difficult one of whether currents in the core can produce and maintain a magnetic field of this character (tied to Ω and to the shape of the crust).

Since it is sensitive to both Ω and the shape of the crust, such a field configuration must be able to slip with respect to the crust. We must therefore postulate the existence of a current sheath at the crust-core interface, which acts to decouple the field in question from the 'radiation' fields within the crust. Such screening currents are likely to appear in the star at the time the crust freezes and we are now studying ways by which this comes about.

We further note that the fields in question need not be dipolar and could well be turbulent; all that is required is that the crustal strains induced by them follow the reference plane. Under these circumstances, equations (1) and (2)

should be replaced by the suitably generalised tensor relations.

Prospects for observing such effects

Assuming that mechanisms for exciting a stellar wobble exist, the amplitude of that wobble will depend, of course, on the extent to which it is damped; here, the situation looks rather favourable for would-be wobble observers. First, in view of the likely homogeneity and sharpness of the stellar surface, the latter a consequence of the strong stellar magnetic field¹⁵, it is reasonable to conclude that no shallow oceans exist on the surface of a neutron star, so that the mechanism responsible for damping the Earth's wobble will be absent. Second, the crustal temperature of a neutron star is exceedingly low compared to its melting temperature ($T/T_m \lesssim 10^{-3}$), so that the only creep mechanism operation is the so-called logarithmic, or low temperature, creep; the time scales for the resulting plastic flow are therefore long compared to the slowing down time, T (ref. 16). Indeed, to the extent that pulsar spindown increases the elastic energy content of the crust, T provides an upper limit to the extent to which plastic flow in the crust can act to damp pulsar wobble.

Crust-liquid coupling is probably the principal source of wobble damping but this too is comparatively ineffective. From the postglitch behaviour of the Crab and Vela pulsars one knows that the time, τ , required for the crust and superfluid neutron liquid to come into equilibrium is of the order of days to years^{17,18}; such coupling gives rise to a damping time for the pulsar wobble which is far longer, being¹⁹

$$\tau_w = \frac{\Omega}{\Omega_w} \tau = \frac{2}{3} \left(\frac{A+B}{B} \right) \frac{\tau}{\epsilon_0} \quad (3)$$

on making use of the expression²⁰,

$$(\Omega_w/\Omega) = \frac{3}{2}(B/A + B)\epsilon_0 \quad (4)$$

for the free precession frequency, Ω_w . For the Crab pulsar, assuming it to have a liquid interior, $(\Omega_w/\Omega) \lesssim 10^{-7}$, so that $\tau_w \gtrsim 3 \times 10^4$ yr, at least an order of magnitude greater than the slowing down time T_{Crab} ($\sim 2,500$ yr); for the Vela pulsar, the wobble damping time is shorter than T_{Vela} ($\sim 24,000$ yr), being ~ 500 yr if the Vela pulsar is assumed to possess a solid core with $\epsilon_0 \sim 3 \times 10^{-3}$.

Given such long wobble damping times, it is reasonable to expect large amplitude stellar wobble for those neutron stars for which the effective magnitude of the exciting torque, L/τ , exceeds the slowing down torque, L/T . In such cases, the amplitude of the stellar wobble is likely limited by the maximum storage of angular elastic energy ($\propto \theta_0^2$ where θ_0 is the angular wobble amplitude) in the solid crust (or core) of the star. For the Crab pulsar, we have previously estimated that starquakes will limit the maximum wobble amplitude for a star of mass $0.25M_\odot$ to some 5° if the angular contribution to the elastic energy is comparatively small, 15° if this contribution dominates the elastic energy¹⁴. For a pulsar with a solid core, comparable wobble amplitudes may be expected; for example, if $\sigma_c/\mu\epsilon_0 \sim 1/7$, the limit on the wobble amplitude is $\sim 6^\circ$.

In their consideration of this problem, Henriksen *et al.*⁶ have assumed that the Goldreich mechanism, in which B_0 is constant in the star, is operative, and have calculated the maximum wobble amplitude for the following two cases:

(1) Stabilisation of the wobble amplitude either by internal friction in the crust or by core-crust coupling.

(2) Wobble at the elastic limit, followed by plastic flow. The estimates we have given above suggest that case (1) is probably not realised in practice; the very low temperature of the crust seems to preclude an effective internal

friction mechanism, whereas for the Crab pulsar, at least, the damping time associated with core-crust coupling is long compared to the excitation time, T , assumed by Henriksen *et al.*, so that core-crust coupling could not act to stabilise the wobble amplitude. Case (2) also does not seem realisable in practice. If only the Goldreich excitation mechanism is operative, examination of the rate of change of the oblateness and angular mismatch terms in the elastic energy shows that the oblateness mismatch contribution will grow at a faster rate than that associated with the wobble amplitude; as a result the wobble amplitude could never be large enough to induce either plastic flow or a microquake. Finally, should it reach such a critical size, we would argue that because the crust is so cold, a series of microquakes are more likely to ensue than plastic flow.

We conclude by emphasising that proposed mass and structure assignments for the Crab and Vela pulsars put definite limits on the precession frequencies one would expect to observe. Thus, for the Vela pulsar, assuming it to be sufficiently massive to possess a solid core, the corequake interpretation¹³ of its giant speedups requires an $\epsilon_0 \gtrsim 10^{-3}$; taking $B \gtrsim A$, we are led to a wobble period

$$P_w \lesssim 100 \text{ s (Vela pulsar)} \quad (5)$$

For the Crab pulsar, assuming it to have a liquid interior and a mass $> 0.3M_\odot$, the wobble period is

$$P_w \gtrsim 16 \text{ h (liquid core Crab pulsar)} \quad (6)$$

On the other hand, assuming the stellar structure of the Crab pulsar were such that it had a solid core and $(I_n/I) \sim 0.9$, one would expect that $\epsilon_0 > 10^{-3}$ and $B > A/10$, in which case

$$P_w \lesssim 5 \text{ min (solid core Crab pulsar)} \quad (7)$$

Observation of a Crab pulsar wobble could thus provide a decisive determination of its stellar structure.

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Relative positions of the 'repetitive', 'unique' and poly(A) fragments of mRNA

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The mRNA molecules from Xenopus laevis embryos represent polyribonucleotide chains of heterogeneous size transcribed from unique DNA sequences, each carrying a poly(A) stretch at the 3' terminus and at the 5' terminus, a fragment of 50 to 60 nucleotides transcribed from repeated DNA sequences.

We have previously discussed the existence of sequence heterogeneity in the mRNA molecules isolated from *Xenopus* embryos¹. On the basis of hybridisation kinetics, we concluded that each mRNA molecule was composed of a main fragment transcribed from unique DNA, with a covalently linked smaller fragment transcribed from homologous repeated DNA sequences². It was, however, impossible to draw any conclusions about the positions that such fragments could have within each mRNA molecule.

As very similar 'repetitive' fragments seemed to be linked to different unique parts, we decided to investigate in detail the uniformity of their size and location in each mRNA molecule, also with respect to the poly(A) stretches known to be present on the majority of mRNAs.

We here present data showing that the 'repetitive' fragment is located at the 5' end of the mRNA and is 50–60 nucleotides long. We have also observed that most of the mRNAs in these embryos contain a stretch of poly(A) which is heterogeneous in size and is located at the 3' end of the molecule.

Isolation of mRNA and characterisation of the poly(A) fragment

Tritium-labelled mRNA was isolated from polyribosomes of dissociated *Xenopus* neurulae following procedures already described^{1,3}. The possible degradation of the mRNAs during extraction was already discussed and ruled out in a previous report¹ by coextracting the embryos with purified globin mRNA. Several controls also showed that significant amounts of labelled newly synthesised rRNA did not contaminate our mRNA preparation. Finally, the possibility that the ³H-uridine counts associated with the polysomes could represent cosedimenting HnRNA molecules leaked out from the nuclei is extremely unlikely in view of the fact that after only 15 min labelling, although a significant amount of newly synthesised RNA was observable, practically no radioactivity could be detected on the polysomes. It was only after longer labelling times (30 min or more) that newly synthesised RNA molecules became associated with the polysomes.

The newly synthesised mRNA sedimented on sucrose gradients as a broad peak of 12S, was very heterogeneous on acrylamide gel electrophoresis and it did not bind to Millipore

filters in 0.5 M KCl (ref. 1). Binding to nitrocellulose filters under these conditions has been reported for poly(A) containing RNAs (ref. 4) and practically all the eukaryotic mRNA investigated (except histone mRNA)⁵ have been found to contain a poly(A) stretch.

As we could have selected for molecules not containing poly(A) by our phenol extraction procedure performed in 0.1 M acetate pH 5.0 (ref. 1), we decided to re-investigate the properties of the mRNA purified from embryos labelled with ³H-adenine. The RNA was phenol-extracted in low salt and neutral pH. In these conditions, more than 90% of the radioactivity present on the polysomes is recovered after extraction and at least 60–70% of the counts are eventually found in the 12S peak on sucrose gradients. The rest of the labelled RNA sediments faster and was usually discarded because of its contamination with 18 and 28S rRNA. Millipore filters retained 25–35% of the RNA purified in this way.

To investigate the content and properties of the poly(A) associated with these RNA molecules, embryos were dissociated and labelled with ³H-adenine for 30 min or 4 h (Fig. 1). The RNA was extracted from the polysomes and digested for 30 min with RNase A and T₁. The amount of RNase-resistant material ranged between 4% and 7% of the total counts incorporated into polysomal RNA. Eighty per cent of the counts resistant to RNase A + T₁ became TCA soluble when digested with a mixture of ribonucleases T₁ + T₂, known to digest poly(A) stretches⁶. Acrylamide gel electrophoresis of the RNase-resistant material shows two distinct patterns; at 30 min (Fig. 1a) the poly(A) is distributed as the predominant peak which migrates slower than the 4S and 5S markers. The average length of the fragments in this peak is more than 120 nucleotides. The counts found consistently on both sides of the main peak demonstrate some degree of heterogeneity in the size of the poly(A) piece. At 4 h (Fig. 1b) a small amount of label is found in the 120 nucleotide peak as the poly(A) pieces are more heterogeneous in size and are distributed towards the lighter region of the

TABLE 1 Elution pattern of RNA and single stranded DNA from the HAP column before hybridisation

Phosphate molarity	% RNA eluted	% DNA eluted
0.12 M	70	100
0.14 M	13	—
0.16 M	13	—
0.20 M	—	—
0.50 M	4	—

500 µg of DNA were denatured in 0.12 M phosphate buffer for 15' at 100°C; during the cooling in ice 5,000 c.p.m. of neurula mRNA were added. The sample was then fractionated on a HAP column at 66°C.

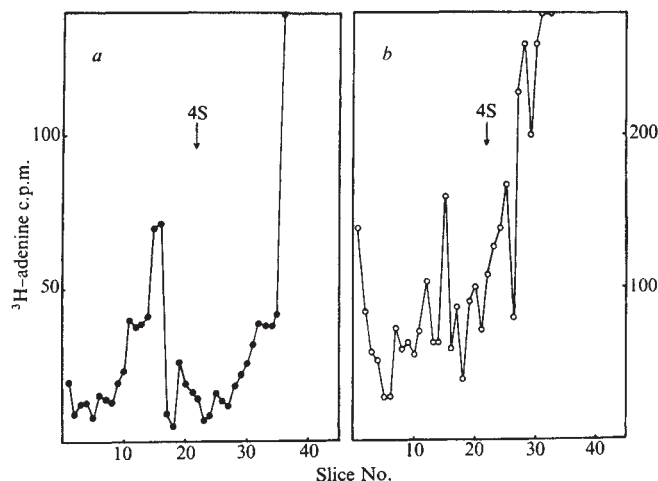


FIG. 1 Determination of poly(A) size. One thousand embryos were dissociated under the usual conditions¹. Half were then incubated in the presence of 100 $\mu\text{Ci ml}^{-1}$ of ^3H -adenine for 30 min (a) while the rest were incubated under the same conditions for 4 h (b). At the end of the incubation the embryos were homogenised in sucrose-TKM buffer (35 mM Tris pH 7.4, 10 mM MgCl_2 , 70 mM KCl, 0.25 M sucrose, 7.5 $\mu\text{g ml}^{-1}$ PVS and 0.5% DOC) and the polysomes prepared by sedimentation on 15–50% sucrose gradients. After extraction of the polysomes with phenol saturated with 10 mM Tris pH 7.5 and chloroform, the two samples were ethanol precipitated. The RNA was resuspended in 10 mM Tris pH 7.5, 0.2 M NaCl and incubated with 20 $\mu\text{g ml}^{-1}$ of RNase A and 10 units ml^{-1} of T_1 for 30 min at 30° C. After ethanol precipitation using 1.0 absorbance unit of *E. coli* soluble RNA as carrier, the RNA was resuspended in 0.5% SDS and electrophoresed in 10% SDS acrylamide gels for 3 h at 5 mA per gel. The gels were cut into 2 mm slices, solubilised and counted. The top of the gel corresponds to the left side in the figures.

gel. Most of these fragments are lighter than the 4S marker and therefore not long enough to retain a whole RNA molecule when passed through Millipore filters. This could explain the low percentage of binding to filters that we obtain with the purified RNA.

We conclude that at short labelling times the mRNA molecules that reach the cytoplasm carry a rather long and homogeneous fragment of poly(A). When longer incubations are performed, the size of the poly(A) fragment decreases and mRNA molecules with shorter heterogeneous poly(A) fragments are detected, which seem to accumulate on the polysomes. Similar observations, which substantiate the cytoplasmic accumulation of mRNA-bearing short poly(A) stretches, have been made using the drug 3-deoxyadenosine³, known to inhibit the synthesis of poly(A) (ref. 7). There are data showing that the cytoplasmic poly(A) has a very wide distribution of sizes⁸ and that poly(A) is metabolised in the cytoplasm^{3,9,10}.

The presence of a poly(A) fragment on each mRNA molecule has also been studied, indirectly, by the use of 3-deoxyadenosine. A batch of dissociated embryos was treated for 30 min before the addition of radioactivity with 50 $\mu\text{g ml}^{-1}$ of 3-deoxyadenosine. The sample was then incubated in parallel with an untreated control for 2 h in the presence of 100 $\mu\text{Ci ml}^{-1}$ of ^3H -adenine. The embryos were homogenised and the polysomes prepared on sucrose gradients. Only 20% of the counts in the control polysomes were found in the 3-deoxyadenosine-treated sample. These results, obtained after long treatment with 3-deoxyadenosine, cannot be due to a specific decrease in RNA synthesis caused by drug damage to the embryos, since embryos kept as long as 2 h in 50 $\mu\text{g ml}^{-1}$ of 3-deoxyadenosine and pulse-labelled with ^3H -uridine for 15 min at different times during this period showed the same amount of incorporation as the controls kept in normal saline solution. Therefore, the ap-

pearance of most of the mRNA on the polysomes must depend on the addition of poly(A) to the messenger precursor.

The poly(A) piece has been reported to be localised at the 3' end of the RNA molecule in many of the messages isolated¹¹; so we decided to determine its exact location in our system, to present a more accurate picture of the possible structure of the mRNA molecules.

For this purpose, the isolated poly(A) piece, obtained as indicated in Fig. 1, was subjected to alkaline hydrolysis in conditions that lead to at least 95% hydrolysis of the polymer to the 2', 3'-monoesters¹². The hydrolysate was then examined by paper chromatography using cold adenosine and synthetic poly(A) (also digested with alkali) as markers for nucleosides and mononucleotides. We found the radioactivity located mainly in two spots (Fig. 2). As expected, the highest counts were found in the region corresponding to mononucleotides and approximately 1% of the total counts were found in the region coinciding with the adenosine marker.

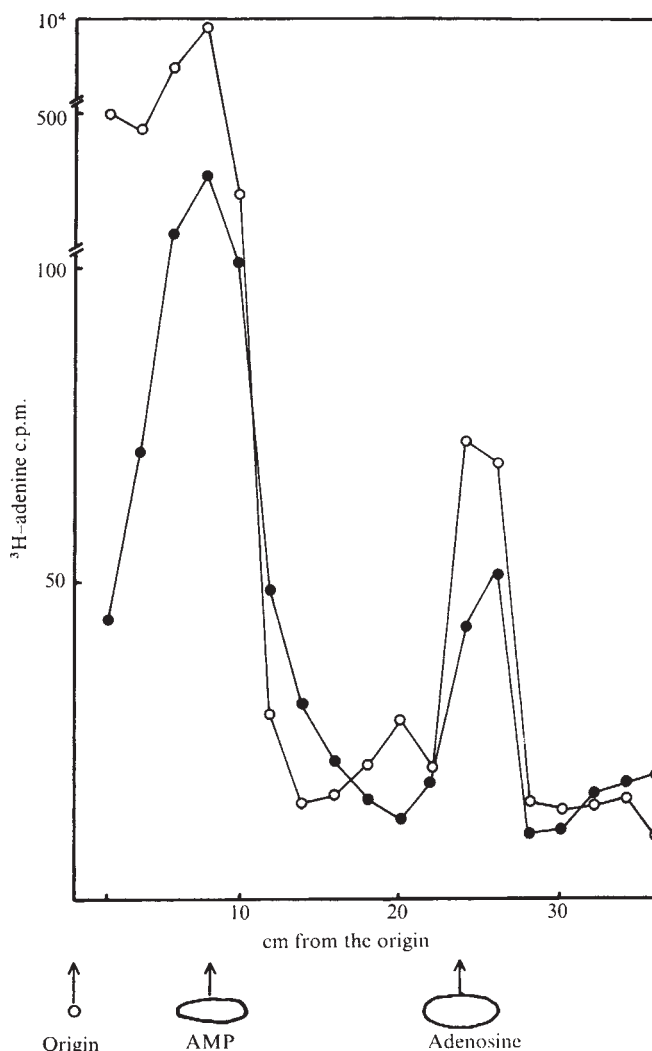


FIG. 2 Distribution of radioactivity in the alkaline hydrolysate of the poly(A) piece. The isolated poly(A) piece, obtained from embryos labelled for 2 h with ^3H -adenine, and synthetic poly(A) (Miles Laboratories, U.S.A.) were hydrolysed for 40 min at 100° C in 0.05M KOH. After hydrolysis the samples were neutralised, spotted on Whatman 3MM paper and subjected to descending chromatography in 1 M ammonium acetate pH 7.5–95% ethanol 30:75 v/v for 10 h. A parallel paper strip containing cold adenosine was run at the same time. Paper strips were cut into 2 cm squares after exposing the spots in ultraviolet light. The paper pieces were counted in Triton-fluor mixture after digestion with 0.1M NaOH for 2 h and neutralisation with acetic acid. ●, poly(A) hydrolysate from the embryo mRNA; ○, hydrolysate from synthetic poly(A).

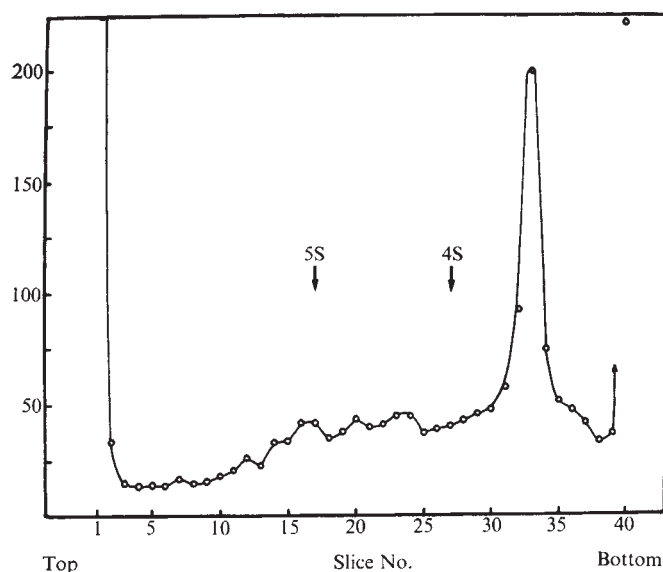


FIG. 3 Size of the isolated 'repetitive' fragment. The RNA (3,000 c.p.m.), obtained as outlined in the text, was resuspended in 0.5% SDS denatured at 100° C and electrophoresed for 3 h on 10% acrylamide gels²² tRNA and 5S RNA were used as markers. Gels were scanned in a Gilford densitometer at 260 nm and sliced into 2 mm pieces. After solubilisation in Soluene, the samples were counted for radioactivity.

The conditions used for hydrolysis do not produce significant dephosphorylation of nucleotides¹² so the nucleoside found should correspond to the 3'-OH terminal nucleotide in the poly(A) stretch. Since the enzymatic treatment does not produce a free 3'-OH end in the nuclease-resistant piece, the free OH group should correspond to the 3' end in the RNA molecule and therefore the poly(A) fragment should be localised at this end. The ratio of labelled nucleotides to nucleosides provides an average value of 75-100 nucleotides per each terminal adenosine residue in the poly(A) stretches. This value is in good agreement with the estimation from gel electrophoresis for the size of the poly(A) fragments from embryos labelled during 2 h (ref. 3).

Isolation of the 'repetitive' RNA fragment

As already mentioned, each mRNA molecule is internally heterogeneous: a part of it is transcribed from homologous repeated DNA sequences while the other is transcribed from the unique part of the genome. These two parts, called 'repetitive' and 'unique' fragments, are covalently linked and therefore present on the same mRNA molecule.

The arrangement of the sequences that we have proposed leads to two predictions: first, if each molecule carries a very similar or even identical 'repetitive' fragment, the size of the isolated part should be somewhat homogeneous. Moreover, the isolated and purified 'repetitive' stretch should rehybridise in the presence of DNA in excess, with its reiterated DNA complements reassociating completely before C_{ot} 100 (see ref. 1).

To isolate the fast hybridising part of the mRNA 120,000 c.p.m. of purified total mRNA was incubated with 30 mg of sonicated denatured DNA to C_{ot} 100. The conditions used were 0.12 M phosphate buffer and 68° C. At the end of the incubation an aliquot was withdrawn and treated with RNase (5 μ g ml⁻¹ RNase A, 30 min at 30° C). The sample was then diluted with 0.12 M phosphate buffer containing 1 M NaCl and loaded on a column of hydroxylapatite (HAP) at 66° C (ref. 13). The ratio of DNA to HAP was about 10 absorbance units of DNA (measured on native DNA before the initial melting) per ml packed HAP. The column was washed with 0.12 M phosphate—1 M NaCl until no

more DNA or radioactivity could be eluted. This step, as shown in Table 1, allows the recovery of about 70% of the RNA counts in the control, monitored before annealing. The column was then washed with increasing concentrations of phosphate buffer, and at 0.16 M the final recovery of the control RNA was 96% of the input. After annealing to C_{ot} 100 the 0.12 and 0.16 M pooled fractions containing the single-stranded DNA and the free RNA accounted for 60% of the DNA loaded and for only 5% of the mRNA counts. The 0.5 M phosphate eluate contained the renatured DNA and practically all the RNA counts. The overall yield of the column was close to 100%.

The behaviour of the RNA on the HAP column reproduces perfectly what has been obtained with nitrocellulose filters¹. The retention of the RNA by the column was 95% while the percentage of real RNase-resistant hybrids was only about 20%. We have already shown that this result is not due to artefacts or to extensive base mispairing, as the melting curves of these hybrids are very close to those of native *Xenopus* bulk DNA¹.

The most likely interpretation of this result is that the entire RNA molecule is retained because a small part of it is able to hybridise to repetitive sequences. The 0.5 M eluate was dialysed extensively against H₂O and then 0.1 M Na acetate pH 5. After ethanol precipitation the sample was resuspended in 2 $\times$ SSC and treated with RNase to eliminate the tails of the non-hybridised 'unique' RNA. Twenty per cent of the counts were recovered and incubated with 100 μ g ml⁻¹ of pronase followed by phenol-chloroform extraction to eliminate the proteins. The DNA/RNA hybrid was concentrated, denatured by heating for 15 min at 100° C and diluted several times by addition of ice-cold 6 $\times$ SSC. The denatured DNA was trapped on nitrocellulose filters while the free RNA fragments were recovered in the filtrate. After a short dialysis to eliminate most of the salt, the RNA was ethanol-precipitated and resuspended in different buffers according to the experiment to be performed.

The final sample contained about 50% of the initial RNase-resistant counts and about 5% DNA not retained by the Millipore filters. The purified RNA fragment was then hybridised to DNA in excess. The amount of DNA added to the reaction, together with the radioactive RNA, was about 10% of the total DNA used in each assay and therefore too low to affect the kinetics of the RNA/DNA hybridisation greatly.

The limited amount of radioactive fragments we could recover from a single mRNA preparation raised serious technical problems when we tried to measure the reassociation kinetics of the purified 'repetitive' stretch. The number of increasing C_{ot} values that could be obtained was too low to make significant measurements of the actual rate of reassociation.

Our results clearly indicate, however, that at C_{ot} 100 all the RNA input was RNase-resistant, from which we conclude that the fragments had completely and rapidly reformed hybrids with the complementary repetitive DNA sequences. These results confirm that repetitive, homologous DNA sequences are transcribed into mRNA molecules which carry also the transcript of heterogeneous unique sequences. Moreover, the 'repeated' RNA fragment can be isolated from the ' C_{ot} 100' hybrids, suggesting that the retention of mRNA by Millipore filters and HAP columns is mediated by the formation of a DNA/RNA hybrid between a family of repeated sequences and a complementary RNA fragment.

Size of the repetitive RNA fragment

The size of the isolated fragment has been measured by electrophoresis on 10% acrylamide gels. Fig. 3 shows the pattern obtained using 5S and transfer RNA as external markers; we estimated an approximate length of 50 to 60 nucleotides for the RNA stretch.

A very small amount of heterogeneous heavier material is also observed but the main radioactivity peak seems fairly homogeneous; two conclusions can thus be drawn. First, the matching of the hybrids (also reflected by the melting curves¹) must be good since RNase cannot cut within this region. It is however possible that the original hybrid might be larger than observed here and so the estimated length must be considered a minimal value. Second, the homogeneity of the fragment, as judged by electrophoretic migration, refers only to the size of the repeated RNA sequences in the formed hybrids. Taking into account the heterogeneity of the filter-binding curve¹, we cannot exclude that more than one class of repeated sequences could be transcribed into fragments of final homogeneous length.

Rapidly hybridising fragment at the 5' terminus of the mRNA molecule

To understand the biological meaning of the observed sequence arrangement in mRNA molecules it is relevant to know where the 'repetitive' fragment is located on the molecule. Since it is known that poly(A) is localised at the 3' end of the molecule, it has been possible to show that the repeated fragment is not associated with this end of the RNA.

The isolated 'repetitive' fragment is too short and homogeneous to account for the presence of any poly(A). Also, if the ³H-adenine labelled RNA is incubated with excess DNA to a C_0t 100, RNase-treated and fractionated on HAP most of the poly(A) is found in the flow through of the column, while none is associated with the retained hybrids³.

Since neither of these experiments can exclude the possibility that the RNase A could introduce a specific cut between the protected hybrids and the free poly(A) fragment, we thought it desirable to obtain direct positive evidence about the location of the rapidly hybridising fragment.

A total population of mRNAs was labelled *in vitro* with ³²P using a technique which allows specific labelling of the 5' end of the molecules^{14,15}. As all the mRNA preparations are slightly contaminated by cold tRNA and rRNA, which will also be labelled *in vitro*, a competition system was set up to prevent the hybridisation of these RNA species³. It has been shown that in excess DNA the hybridisation of trace amounts of hot RNA can be efficiently competed with by a five-fold input of cold RNA of the same species^{16,3}. The residual hybridisation obtained under these conditions is about 10%. Our experiments with ³²P-labelled RNA have been run in the presence of fifty-fold excess cold oocyte RNA. Under these conditions the hybridisation of these RNA species to a given amount of DNA (see legend of Fig. 4) should be completely prevented without causing any interference with the reassociation of the mRNA.

Figure 4 shows that when a total population of mRNA is hybridised with excess DNA¹⁷ most of the RNA hybridises to unique sequences. Only about 20% of the RNA rapidly hybridises with repeated sequences. This result confirms that the hybridisation pattern obtained in previous experiments¹ was not due to a qualitatively selected population of mRNA. In fact, the only difference we have been able to detect between total mRNA and RNA extracted with phenol at pH 5 is that the latter seems to be enriched with molecules carrying shorter poly(A) fragments.

If the same batch of mRNA is labelled *in vitro* at the 5' end and the hybridisation in excess DNA is performed (Fig. 4) monitoring the ³²P RNase-resistant counts, a completely different pattern is obtained. All the counts are hybridised at C_0t 100 and the reassociation follows second order kinetics. This should be indicative of a single reassociating species with a $C_0t_{1/2}$ of about 12–25, which in our conditions is slightly faster than rRNA. We therefore conclude that a fragment, transcribed from a family of sequences with a reiteration of about 2,000 copies per diploid genome

is present on polysomal mRNA at the 5' end of the molecule.

Previous results obtained with the filter trapping technique^{1,16}, were indicative of more than one fast renaturing component. In fact about 30% of the RNA was retained by the filters at a very low C_0t (10^{-1}) while the rest of the reaction was following slower kinetics.

The homogeneity of the hybridisation kinetics obtained with the ³²P-labelled RNA may be explained in two ways. The population of RNAs used for the filter trapping experi-

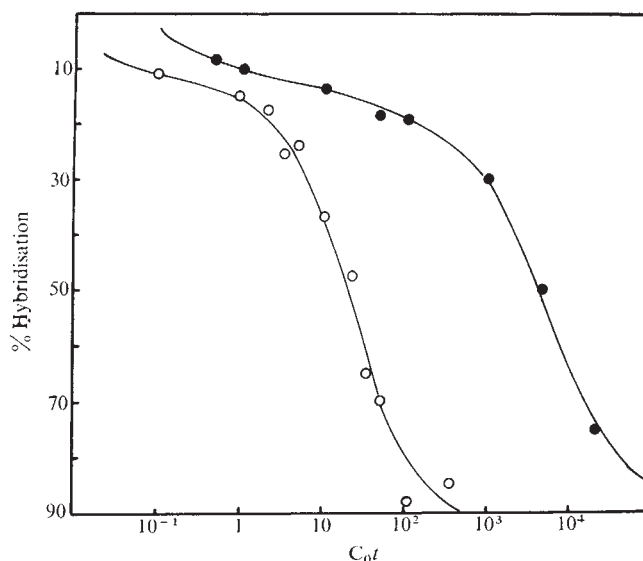


FIG. 4 DNA excess hybridisation of ³H-adenine labelled mRNA and 5' ³²P-labelled mRNA. The ³H-labelled mRNA was prepared from polysomes of 4 h labelled embryos as specified in Fig. 1. The hybridisation was performed in a great excess of DNA¹. A blank value equal to 5% of the input has been subtracted at each point as this was the RNase A resistance of the purified mRNA due to the presence of labelled stretches of poly(A). The same batch of ³H-labelled mRNA was then labelled with ³²P according to the procedure of Ilan and Ilan¹⁴. ³²P-2-cyanoethylphosphate pyridinium salt was prepared according to the method of Pfizner and Moffat¹⁵ (modified to obtain a specific activity five times as great), and was used as a donor molecule. The mRNA was treated with 10 μ g ml⁻¹ of alkaline phosphatase for 1 h at 30° C in 0.1 M Tris pH 8.2, to cleave the 5' end phosphate. To avoid degradation of mRNA, and subsequent formation of artefactual free ends, a nuclease-free preparation of enzyme was used (Boehringer, Mannheim). After phenol and chloroform extraction, the RNA was precipitated in ethanol and resuspended in dimethyl formamide. ³²P-cyanoethylphosphate in dry pyridine was added (15 ml) and the sample evaporated to dryness at room temperature. Dicyclohexylcarbodiimide (2 g) in 15 ml of pyridine was added to resuspend the mixture and the sample was incubated in a stoppered vial for 20 h at room temperature. After drying under vacuum the residue was resuspended in 30 ml water containing 1 mg of *E. coli* tRNA as carrier, centrifuged 30 min at 30,000g and the supernatant was ethanol precipitated. The RNA was resuspended in water adjusted to pH 9 with ammonium hydroxide, and the mixture was heated at 60° C for 75 min to cleave the cyanoethyl group. Before hybridisation, the RNA was further purified on a Sephadex G25 column (1.4 $\times$ 30 cm) to remove the residual free ³²P counts. The peak of radioactivity eluted in the void volume of the column was pooled and ethanol precipitated. The pellet was finally resuspended in 0.12 M phosphate buffer. The resulting RNA sedimented, in sucrose gradients, close to the 4S marker. The yield of ³H counts was equal to about 50% of the input and the yield of ³²P was about five times greater. All the ³H and ³²P counts of this final RNA fraction became completely soluble in TCA after either alkaline hydrolysis or RNase treatment. Alkaline phosphatase treatment removed only the ³²P label. Excess DNA hybridisation was performed under the usual conditions but to obtain reproducible blanks (20–30 counts) every sample was extracted with phenol after the RNase treatment. The samples were then TCA precipitated and counted. The curve is the average of two separate experiments.

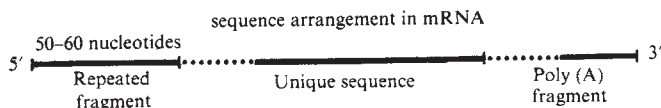


Fig. 5 A schematic mRNA molecule.

ments was extremely heterogeneous with a range of sizes varying between 6 and 18S (ref. 1). The reassociation rate of a family of sequences is strictly correlated to their size¹⁹, therefore the heterogeneous pattern of filter trapping we observed might be due to the presence of this wide variation in the size of the reassociating RNA molecules. This size effect is completely excluded using the ³²P-labelled RNA, as during the cleavage of the cyanoethyl group (see legend of Fig. 4) all the RNA is degraded to a size slightly smaller than 4S. The final hybridisation rate obtained with these fragments is slower than that for the original ³H-labelled large molecules and the homogeneity of the curve probably results from the final common length of the fragmented RNA.

Also, although most of the molecules carry a repetitive stretch at the 5' end some of them may have a second, faster hybridising part at a different position in the molecule. When the curve is monitored by the retention of hybrids on nitrocellulose filters¹, this second repetitive stretch could also hybridise to its DNA complements leading to the trapping of the entire RNA molecule, giving a final heterogeneous pattern of retention. This feature cannot be observed when the hybridisation is monitored by following the RNase-resistant ³²P counts, as in this case only the fragment at the 5' terminus is labelled.

The specificity of the cyanoethylphosphate *in vitro* labelling technique must be considered. In agreement with Ilan and Ilan⁴, after alkaline hydrolysis and DEAE-Sephadex chromatography we recovered, as expected, most of the ³²P counts as pXp. The presence of a small amount of monophosphates (and therefore of 3' labelling) cannot be completely excluded on the basis of this experiment since it is very difficult to purify the RNA from all the free ³²P counts which elute from the DEAE-Sephadex column in the monophosphate region. Significant labelling of the 3' end carrying the poly(A) fragment, however, does not occur, since 90% of the ³²P counts are digested by RNase under conditions which leave the poly(A) stretches intact.

The alkaline phosphatase stage in the procedure might cause some breakage of the mRNA before the *in vitro* labelling. This produces artefactual 5' ends where a phosphate could be introduced during the *in vitro* labelling procedure. The hybridisation pattern expected here, however, would be similar to the one obtained with *in vivo* ³H-labelled mRNA. It can be calculated that with only two randomly introduced breaks per molecule, approximately 70% of the *in vitro* labelled RNA would be expected to behave as 'unique'. This is certainly not the case (Fig. 4) and therefore our results clearly show that significant breakage cannot have occurred during the alkaline phosphatase treatment unless specific cuts were introduced only in the regions containing the 'repetitive' fragment. We have no way to check this possibility at present, because of the size heterogeneity of our mRNA population.

Sequence arrangement on mRNA

Figure 5 shows a diagram of an mRNA molecule based on our experimental results. Every molecule of a heterogeneous population of mRNAs is made up of three main parts. At the 5' end there is an homologous fragment, 50 to 60 nucleotides in length, transcribed from repetitive sequences. This fragment is present on every molecule and is covalently linked to a longer heterogeneous sequence transcribed from the unique part of the DNA. In agreement with reported data

for different kinds of cultured cells^{11,10} we also found evidence that poly(A) is probably added post-transcriptionally at the 3' end of the molecules. Ilan and Ilan¹⁴ reported the existence of a short homologous fragment at the 5' end of insect mRNAs; this suggests that the sequence arrangement we have observed might be a common feature of eukaryotes.

In this respect, it is relevant that experiments done directly on DNA have shown the existence of a pattern of sequence distribution which could give rise to the transcriptional products we observed. Davidson *et al.*²⁰ reported that at least 50% of the genome of *Xenopus laevis* is organised with short (300 nucleotides average) repetitive sequences intermingled with longer (1,000 nucleotides average) unique sequences. The rest of the genome also seems to be organised similarly, but with longer unique sequences interposed. As in this system, few of the intermediate steps of the mRNA maturation in the nucleus are known, it is impossible to make any strict correlation between the sequence sizes observed on the RNA molecules and the described structure of the genome.

The biological roles of the different RNA sequences carried by each molecule are so far unknown, except for the 'unique' part, which supposedly carries the information for protein synthesis. It seems important, however, that the repetitive fragment on cytoplasmic mRNA be in a specific position (5' terminus). It is possible that this sequence plays a role at the level of mRNA maturation in the nucleus and is possibly the result of the partial cleavage of one of the 'hair-pins' known to exist in HnRNA (ref. 21). A more attractive suggestion is that the homologous sequences on different messengers could be involved in translational control mechanisms.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Radio source identified with a neutral stellar object near an unusual galaxy

RECENT improvements in the accuracy of location of radio source positions to 1 arc s have made possible the reliable identification of a number of radio sources with stellar objects of neutral colour (NSO)¹⁻⁴. Two of these have the highest known redshifts, 3.4 (ref. 5), and 3.53 (ref. 6). Others have featureless optical spectra and are probably BL Lac objects⁷ (Carswell *et al.*⁸ and P. A. Strittmatter, private communication). We report here the identification of an NSO of 19 mag close to an unusual blue galaxy, using an accurate radio position obtained at 408 MHz with the Mills Cross of the Molongo Observatory.

The radio source 1107+036 was reported by Munro⁹ and has been identified with a nearby blue galaxy of 17 mag¹⁰ (for reasons explained below) rather than with the NSO of 19 mag noted to the west and closer to the radio position. Figure 1 is an enlargement of a section of prints from the National Geographic-Mount Palomar Sky Survey, which clearly shows both objects. The radio positions in ref. 10 were accurate to ~ 5 arc s and the total number of NSOs within the radio error regions was consistent with chance expectation. Radio and optical positions accurate to ~ 1 arc s are necessary to identify an NSO reliably. A position of this accuracy has now been obtained at Molongo for 1107+036. The improvement principally arises from repeated observations during special calibration sessions, in which a large number of sources with known optical positions are observed on successive days, and other objects of interest are interspersed with these. The main purpose of these sessions is to discover and monitor time-varying sources¹¹ and to test the performance of the aerial.

The radio source 1107+036 was observed on six different days spanning three different calibration sessions. The use of numerous calibrators close to the source and the repeated observation of the source enables the rejection on statistical grounds of occasional results affected by severe ionospheric or meteorological conditions. The result is presented in Table 1 together with the optical position obtained from the sky survey prints. A new optical measurement for the NSO is consistent with that already reported¹⁰ and the mean of the two measurements is given in Table 1.

The identification is highly likely because the probability of a chance association at a separation of 2.1 arc s is about 0.004, based on star counts¹⁰ in the appropriate range of galactic latitude.

The source does, however, differ in one important respect from most NSOs identified with radio sources, in that it has a steep radio spectrum with spectral index 0.99 ± 0.07 . This is a revision of the value quoted by Murdoch and Hoskins¹² and uses the present flux density at 408 MHz of 1.77 ± 0.05 Jy (1 Jy = 1 f.u.). Extrapolation to 178 MHz would indicate at flux density of 4 Jy but the source does not appear in the 4C catalogue¹³. This could indicate self absorption of low frequency but is probably a result of the nearby occurrence of 1108+034 (4C03.21) and 1107+045 (4C04.35). Most radio sources identified with NSOs have 'abnormal' radio spectra¹⁻⁵, (where the term normal implies a reasonably

straight radio spectrum with spectral index steeper than -0.5). It is not clear, however, to what extent the predominance of abnormal spectra may be a selection effect which arises because most of the accurate positions necessary for the identification of NSOs have been obtained at high frequency.

The nearby blue galaxy is an interesting object in its own right. There is an extensive blue envelope of diameter ~ 20 arc s which is apparently non-uniform. The most prominent features are a blue condensation to the north and, on the red print, a curved feature extending outwards from the central region toward the south-east. A high resolution photograph is necessary to test the reality of these features.

The original identification was made with the galaxy¹⁰ despite its displacement outside the error region, because it was found that the total number of galaxies beyond the radio error regions, but within 20 arc s of the respective radio positions, was significantly in excess of chance expectation. Among the various cases in this category, 1107+036 seemed to be one of the more likely cases of a genuine displaced identification. Now that an alternate identification has been found, the question arises as to whether the nearby presence of the galaxy should be regarded as coincidental, or whether the two objects might be associated.

We might question whether all of the galaxy identifications suggested by Hoskins *et al.*¹⁰ with radio-optical separations greater than the error limits might be coincidental. This probability was assessed by them to be 0.007. We note incidentally that Véron and Véron¹⁴ have suggested that radio-optical separations are very rare. There is, however, further supporting evidence for such radio-optical separations for galaxies when measurements are made with the 2.9-arc min beam of the Mills Cross. Separations up to ~ 30 kpc were common for galaxies in Abell clusters¹⁵. Separations of ~ 40 kpc from the radio centroid have been found for galaxies with low-brightness extended components¹⁶. We do not, therefore, believe that all the suggested identifications involving a radio-optical separation should be rejected.

There are two sources apparently similar to 1107+036 that warrant brief mention. The variable radio source 0048-097 has recently been identified⁴ with an NSO, in place of the original identification with a nearby blue galaxy¹⁷, and is

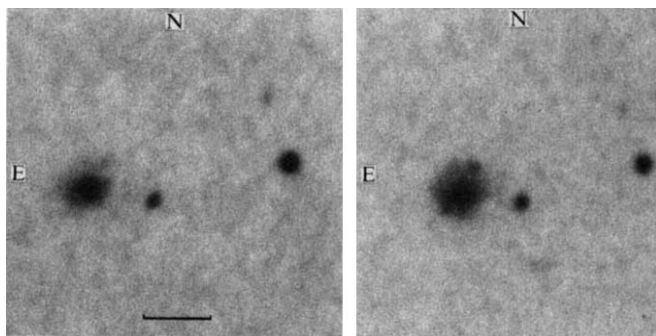


FIG. 1 Red and blue images of the NSO 1107+036 and the nearby galaxy, enlarged from the National Geographic-Palomar Survey prints. The scale marked on the red print is 20 arc s long.

TABLE 1 Molonglo positions for NSO1107 + 036

Radio	408 MHz	11 h 07 min 49.38 $\pm$ 0.07 s	03°37' 52.8 $\pm$ 1.5"
Optical	NSO	11 h 07 min 49.25 $\pm$ 0.03 s	03°37' 53.6 $\pm$ 0.4"
	Galaxy	11 h 07 min 50.51 $\pm$ 0.04 s	03°37' 56.3 $\pm$ 0.6"

probably a BL Lac object⁷. In this case the NSO is brighter than the galaxy. The second case is 0837+242 (4C24.18) which was identified with a nearby irregular blue galaxy (17 mag, ref. 18) rather than with a stellar object closer to the radio source. Burbidge¹⁹ found a continuous optical spectrum for the stellar object which has been described as blue^{14,18} and as neutral¹⁹. Véron and Véron¹⁴ consider the stellar object to be the identification but J. M. Sutton (private communication) has obtained a more accurate radio position which is 13 arc s from the stellar object. The identification is therefore seriously in doubt.

The one additional case of an NSO within 20 arc s of a blue galaxy is not a convincing argument for associating the blue galaxy with the radio source in either case (0048-097 or 1107+036). In the absence of direct evidence, we consider that further cases need to be found before the association can be justified statistically.

The general nature of neutral stellar objects is not clear, but it is suspected that they are linked to the BL Lac objects (Carswell *et al.*⁸, and P. A. Strittmatter, private communication). Racine²⁰ has suggested that BL Lac (and hence presumably other objects in this class) may represent a particular type of galaxy closely related to N galaxies and Seyfert galaxies. Among other bright BL Lac objects and possible BL Lac objects 1727+50 (ref. 7) was listed as a very compact galaxy²¹, 1101+38 (ref. 22) was listed as an extremely compact galaxy²¹, and AP Lib (1514-24) was classified as an N galaxy²³.

In fact, a problem in classifying the optical counterparts of radio sources is that any morphological classification depends directly on apparent magnitude (and therefore, presumably, distance). It may well be that the NSOs currently being identified are fainter members of the same class of compact objects. The occasional nearby presence of other galaxies would then no longer be surprising because of the known tendency of galaxies to cluster.

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Search for high frequency optical variations in Vela XR-1

THE binary system associated with the 6.87-mag B0.5Ib star, HD 77581, which is identified^{1,2} with the Vela X-ray source, 2U0900-40, is a good candidate for optical studies with high time resolution because of its relative brightness. A 28-min data set consisting of consecutive integrations of 1 ms was taken on the night of February 25-26, 1973 with the Cerro Tololo 152-cm telescope using an S-20 photomultiplier with no filter. Analyses using power-spectral and cepstrum techniques³ show no significant activity at a level greater than 0.25% in the range from 0.06 Hz to 500 Hz (except for instrumental artefacts at 60, 180, 300 and 500 Hz). No aperiodic variations were detected to a weaker limit (1%-2%); at low frequencies (<10 Hz) increased variance ascribable to atmospheric scintillation was present. This result was confirmed by a separate analysis of about 15 min of data from an observation of 1 h on the night of January 13-14, 1973. That the X-ray emission is actually associated with a degenerate, collapsed (and perhaps invisible) companion to the brilliant supergiant, however, makes a stronger limit very desirable.

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Alaska to New Zealand whistler-mode transmission at 6.8 kHz

OBSERVATIONS of whistler-mode signals from v.l.f. communications transmitters have been used^{1,2} to study the magnetosphere in a way similar to the use of naturally occurring whistlers from lightning. Existing naval communications transmitters provide high radiated power (up to 1 MW) but are restricted to transmission at allocated frequencies mostly above about 15 kHz, are not well located geographically for this work and are not often available to transmit special programmes.

We report here one result of an experiment for which we set up a transportable v.l.f. transmitter in Alaska and a receiving station in New Zealand located so as to be approximately geomagnetically conjugate. Since the Otago group already had v.l.f. receiving facilities at Dunedin, New Zealand, the transmitter was set up (by the Aerospace group) at Port Heiden, Alaska, about 200 km from Dunedin's conjugate. The transmitter antenna was a vertical monopole lifted to 1,000 to 1,500 m by balloon. The transmitter and all associated equipment (including power generator) are mounted on trailers so that the installation can be set up or removed in a matter of days. Although transmitter location and frequency are quite flexible, radiated power obtainable is quite low. This varies from a few kW at 21 kHz to about 100 W at 7 kHz. During the event described here the radiated power was only 13 W.

The event occurred about 1 h after local midnight on (Greenwich date) August 27, 1972. The transmitter operated at 6.8 kHz (at 13 W) from approximately 1307 to 1342 GMT. Whistler-mode signals were first detected at Dunedin at about 1324 GMT. Signal strength increased with some variation to a maximum of $35 \mu\text{V m}^{-1}$, before fading again to below detectability at about 1330 GMT.

During this period of good reception, the transmitter was keyed on for 0.5 s in each second from 1326 to 1327:41 and then continuously ("c.w.") to 1329:10 GMT. Before 1326 and after 1329:10 the transmitter sent pulses of varying length and separation. From these the one-hop delay was measured as 1.13 ± 0.01 s. Maximum signal strength ($35 \mu\text{V m}^{-1}$) occurred between about 1328:20 to 1328:40 during c.w. transmission. A short section is shown in Fig. 1.

Twelve whistlers were received at Dunedin during the period 1325 to 1330. Most reached up to 8 kHz and were strongest between 5 and 6 kHz. Eight of these whistlers were 'short' or one-hop and the other four were 'long' or two-hop. None showed echoes beyond 2-hop, or any associated hiss or triggered emission, or any other evidence that duct amplification might be higher than typical.

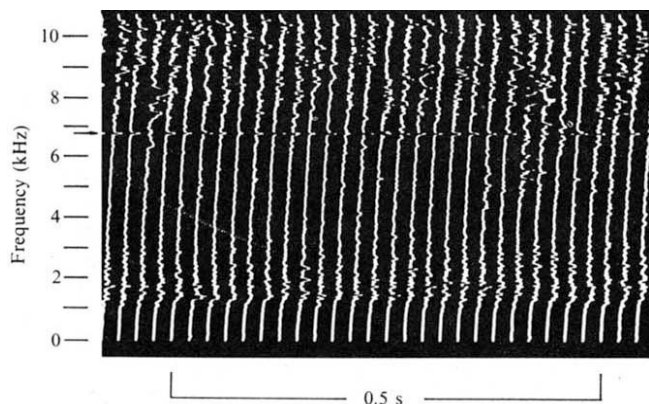


FIG. 1 Dynamic spectrogram of a short section near 1328:30 UT, using a Rayspan scanning at 48 sweeps s^{-1} . Trace separation corresponds to about $40 \mu\text{V m}^{-1}$. Signal at 6.8 kHz is indicated by arrow.

Although no whistler reached the nose frequency, dispersion analysis using the "linear-Q" technique³ showed that all whistlers travelled in the one duct at $L = 2.92 \pm 0.04$ (invariant latitude 54.2°) with one-hop delay at 6.8 kHz of 1.13 ± 0.01 s. From this it can be safely assumed that the signals from our transmitter travelled in this duct also.

An estimate of the duct amplification can be made by comparing the observed signal strength with that calculated for no duct amplification. Helliwell provides analytic expressions and graphs for making such calculations on pages 64 to 81 of ref. 4. Briefly his method involves calculating the field of the wave transmitted into the duct, the effective cross section, trapping and transmission efficiency of the duct, the field at the duct output (Dunedin end), the absorption through the D, E and F region, and finally the waveguide loss from the duct exit region to the receiver (Dunedin). Although we know the duct latitude by dispersion analysis, we do not know duct longitude and hence the distances Port Heiden—duct entrance and duct exit—Dunedin. If the duct were fortuitously located to minimise these distances (100 km and 160 km respectively) the signal expected would be about $1.5 \mu\text{V m}^{-1}$ to within about 3 dB. The observed signal strength of $35 \mu\text{V m}^{-1}$ thus implies amplification of about 27 ± 3 dB for optimum duct longitude. Higher amplification would be needed for other duct longitudes. Thus if the duct exit had been a "reasonable" 1,000 km from Dunedin, an additional 20 dB of amplification would be required.

Since the observed whistlers were typical and unremarkable, we conclude that duct amplification at night is typically of the order of 30 dB per hop. As a consequence whistler mode signals can be obtained from quite low power transmissions at 'low' (~ 7 kHz) frequencies.

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Effect of atmosphere and ionosphere on magnetospheric micropulsation signals

THE large-scale structure of low frequency hydromagnetic waves in the magnetospheric plasma can be understood from observations of micropulsations at the Earth's surface. Signals have to penetrate the ionosphere and atmosphere and although the scale heights of these layers are much less than the scale lengths expected for the magnetospheric phenomena, the behaviour is not as simple as might be expected^{1,2}.

Recent computations³ (also, W. J. Hughes, to be published) allow an analytical approach to the problem.

Dungey¹ split the disturbance seen on the ground into two parts, one part with zero vertical current at the ground and the other with a vertical current. The latter part behaves differently from the former and is in fact strongly screened by the ionosphere. The vertical current at the ground is non-zero and this part of the disturbance therefore has a vertical electric field associated with it, given by

$$\frac{1}{c} (4\pi\sigma + i\omega)E_z = (\text{curl } \mathbf{b})_z$$

where $\mathbf{b}$ is magnetic disturbance and σ is the atmospheric conductivity. Therefore, near the ground

$$E_z \sim \frac{cb_g}{(4\pi\sigma_g + i\omega)l} \quad (1)$$

where l is the horizontal scale length of the disturbance. This vertical electric field is related to the horizontal electric field by Faraday's law so that, also near the ground,

$$\frac{\partial E_y}{\partial z} - \frac{\partial E_z}{\partial y} = \frac{i\omega}{c} b_g \quad (2)$$

where y is the direction of horizontal variation of the micropulsation magnetic field. Using equation (1) in equation (2), together with the approximation

$$\frac{\partial E_z}{\partial y} \simeq \frac{E_z}{l}$$

it is clear that if

$$\frac{(4\pi\sigma + i\omega)\omega l^2}{c^2} \ll 1 \quad (3)$$

then

$$\frac{\partial E_z}{\partial y} \simeq \frac{\partial E_y}{\partial z} \quad (4)$$

and the disturbance is effectively electrostatic in the atmosphere. Equation (3) is satisfied for $\omega \sim 0.1$ – 0.01 s⁻¹ by any non-uniform disturbance on the Earth.

It follows that because the electric current is divergence free and the conductivity rises markedly with height, E_z decreases rapidly with height. Equation (4) shows that the change of E_y with height is proportional to E_z and, as the numerical integrations show, with any reasonable atmospheric model the horizontal electric field is more or less constant from a height above 20–30 km and will be of the order $h E_{zg}/l$, where h is the scale height for conductivity (and thus for E_z).

This electric field drives both Pedersen and Hall currents in the ionosphere and the increase in magnetic field resulting from the currents, in both the x and y directions, is given by Ampere's Law and is of the order

$$\begin{aligned} \Delta b &\sim \frac{4\pi}{c} \Sigma E_y \\ &\sim \frac{4\pi}{c} \Sigma \frac{h E_{zg}}{l} \\ &\sim \frac{4\pi}{c} \Sigma \frac{h}{l} \frac{cb_g}{(4\pi\sigma_g + i\omega)l} \end{aligned}$$

where Σ is a height integrated ionosphere conductivity. For $\Sigma \sim 10^{13}$ electrostatic units (e.s.u.) $h \sim 10^8$ cm, $l \sim 10^8$ cm and $\omega \sim 10^{-1}$ s⁻¹ the field at the ionosphere is $\sim 10^5$ greater than that at the ground. This part of the disturbance is thus effectively screened from the ground by the ionosphere.

The other part of the disturbance, that is, the part with $(\text{curl } \mathbf{b})_z = 0$ at the ground, behaves more simply. Everywhere at the ground $E_z = 0$ and so the horizontal electric field and magnetic field are related by

$$\frac{\partial E_x}{\partial z} = \frac{i\omega b_{yg}}{c}$$

or

$$E_x \sim \frac{i\omega}{c} z b_{yg}$$

Computations show that this approximation is valid up to the top of the ionosphere. The change in magnetic field produced by the ionospheric current is of the order

$$\Delta b \sim 4\pi b_g \frac{\Sigma d\omega}{c^2}$$

where d is the mean height of the high Pedersen or Hall conductivity region in the ionosphere. For $\omega \sim 10^{-1}$ and $d \sim 10^7$ cm, Δb is of the order of the magnetic field at the ground. At lower frequencies ($\omega \lesssim 10^{-2}$) the ionospheric contribution to this part may be ignored, and the magnetic fields above and below the ionosphere are the same.

As the ionosphere screens only that part with $(\text{curl } \mathbf{b})_z \neq 0$, then $(\text{curl } \mathbf{b})_z$ must be effectively zero for any disturbance seen on the ground, whatever the disturbance in the magnetosphere. The magnetospheric disturbance is determined by the hydromagnetic wave equations and boundary conditions very far from the Earth. A magnetospheric disturbance will generally contain parts of each disturbance described, and thus will not be totally screened.

In a cold plasma (which is a good approximation for most of the magnetosphere away from the equatorial region) there are two wave modes; the isotropic fast mode and the transverse mode which is guided by the magnetic field. The scale length of variation for the isotropic mode is $2\pi A/\omega$ where A is the Alfvén speed. For $\omega \sim 10^{-1}$ – 10^{-2} s⁻¹, and an Alfvén speed of the order of 10^8 cm s⁻¹, the scale length of the fast mode is much greater than terrestrial dimensions. This implies that if the disturbance is localised on the Earth, the fast mode contribution is evanescent and the vertical scale of variation of the disturbance in the magnetosphere is comparable to its horizontal scale on the ground. If boundary conditions are then applied between the ionosphere and the magnetosphere for horizontal scales of about 300 km or greater, the disturbance on the ground is proportional to the transverse mode amplitude in the magnetosphere.

Observations of the localisation in latitude of an event at a given micropulsation frequency^{4,5} suggest that the field line resonances of the transverse hydromagnetic mode are an important source of micropulsations.

At mid and high latitudes, however, where the dip angle is large, the transverse mode has $\nabla \cdot \mathbf{b}_{\text{HOR}} \simeq 0$ in the magnetosphere. But on the ground the ionospheric screening ensures that only the part with $(\text{curl } \mathbf{b}_g)_z \simeq 0$ will be seen. If the horizontal scale is great enough for the magnetospheric and ground magnetic fields to be directly proportional, then the ionospheric currents must act so as to rotate the horizontal vector of the magnetic field through 90°. In such a case the horizontal polarisation pattern seen on the ground needs to be rotated through a right angle to obtain the magnetospheric pattern.

Features of a disturbance which exhibits a peak at a fixed latitude and a periodic variation in longitude, can be simply outlined. At the latitudinal peak the disturbance should be linearly east-west polarised on the ground. Elsewhere the ground polarisation will be elliptical with the major axis north-south for events which are strongly lo-

calised latitudinally. This result is in agreement with some earlier experimental observations⁵⁻⁸.

In conclusion, the properties of micropulsation signals discussed here seem to be an important feature of at least one class of pulsations; and our studies suggest that to a first approximation the polarisation ellipse on the ground should be rotated through a right angle to obtain the magnetospheric polarisation.

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Middle Cretaceous sediments from the eastern part of Walvis Ridge

THE Walvis Ridge is one of the most conspicuous features of the South Atlantic. It extends from near Tristan da Cunha on the Mid-Atlantic Ridge to the African continental margin and has three main segments. The western segment is oriented SW-NE, the central one N-S and the eastern one SSW-NNE. The Walvis Ridge may have kept pace with the opening of the South Atlantic which started in the Early Cretaceous¹⁻⁶, either by transform fault mechanisms^{7,8} or by a mantle hot spot and plume⁹⁻¹². The eastern segment

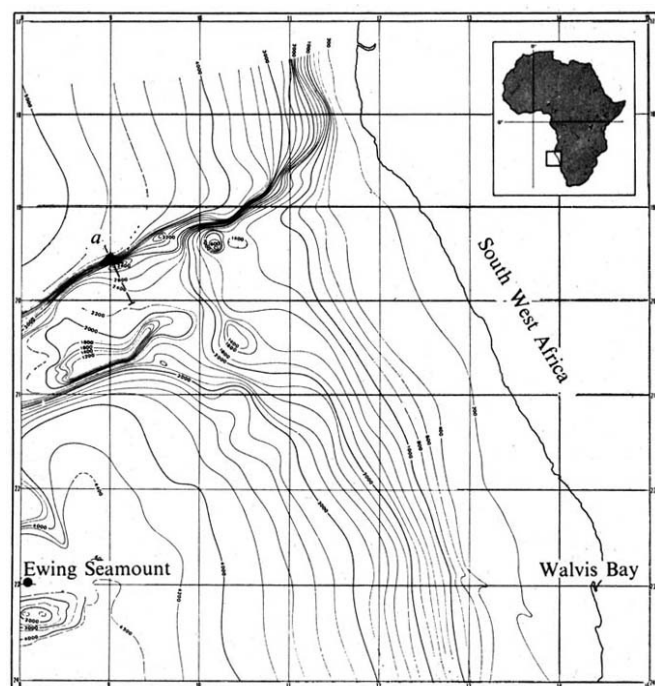


FIG. 1 Bathymetric map of the eastern segment of the Walvis Ridge showing the location of the seismic reflection profile shown in Fig. 2, and the locations of the sediment samples. *a*, Site of dredge. Depths in corrected metres. Contours at intervals of 200 m.

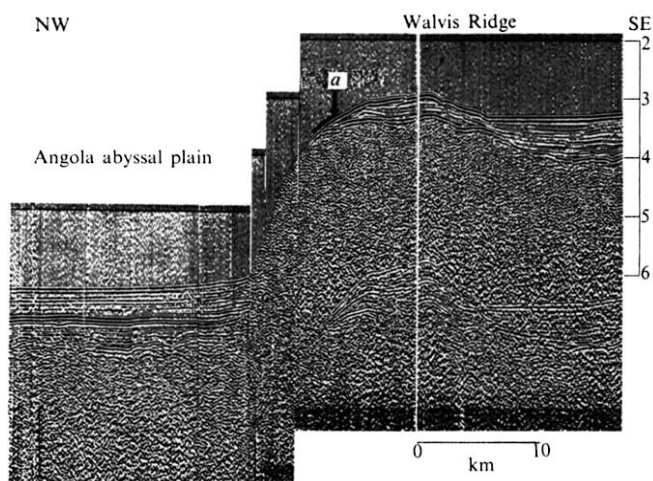


FIG. 2 Seismic reflection profile across the northern scarp of the eastern segment of Walvis Ridge and location of the dredged rocks. *a*, Site of dredge.

is thought to have been built throughout the Middle Cretaceous, that is, between 120 m.y. ago and 80 m.y. ago¹³⁻¹⁵. We report information obtained from an investigation of the age and depositional conditions of sedimentary rock recovered during the Walda cruise of the RV Jean Charcot (April-August 1971).

White chalk fragments containing numerous fossils were recovered from a depth of 2,700 m by a dredge (CH 18 DR 04) on the northern flank of the Walvis Ridge in the centre of the eastern segment (19° 33'S; 09° 01'E) (Fig. 1). Smear slides of the calcareous matrix show mainly coccoliths and micritic calcite. Glauconite and biotite are also present. The most abundant macrofossils are gastropods, remains of echinoderms (regular and irregular urchins: *Holasteroidea*, *Spatangoida*) and bivalves (including *Ostreidae*, *Arcacea*, *Heterodonta* and *Neithea*). One specimen of *Neithea* could be *Neithea cf. shawi* Pervinquier (= *N. coquandi* Péron). The distribution of *N. shawi* is Albian-Cenomanian in the Angola Basin but mostly Cenomanian in northern Africa¹⁶. This specimen is, however, poorly preserved and could belong to the species *N. regularis* Schlotheim. In Europe the distribution of *N. regularis* Schlotheim ranges from Turonian to Senonian (Freinex, personal communication). Thus the macrofossils indicate an Albian-Senonian age. The nannofossils consist mainly of *Lithastrinus floralis* Stradner, *Cricololithus multiradiatus* Kamptner, *C. pennatoideus* Deflandre, *Loxolithus armilla* (Black) and *Parhabdololithus embergeri* (Noël). These are typical Albian-Cenomanian species. The sediment is therefore tentatively dated Middle Cretaceous, that is, about 100 m.y.BP. This agrees with ages proposed by other authors¹³⁻¹⁵.

Macrofossils are not common in a coccolith ooze. Hypotheses for a depositional environment of the sediments, which might explain the observed association include (1) the coccoliths accumulated in deep water and the macrofossils are thanatocoenoses transported from shallow depths by turbidity currents or some other process; (2) both the coccoliths and macrofossils were deposited in shallow water. We favour the second hypothesis, tentatively rejecting a mixed origin for the sediments because of the distance from the African coast and the absence of local seamounts. A shallow water environment would comply with the ecological needs of the macrofossils. *Neithea*, for example, is commonly found in shallow water Albo-Cenomanian deposits in the Angola Basin¹⁶. Furthermore, coccoliths are epipelagic organisms and cannot be used as precise depth indicators¹⁷. Although they are typical of open sea sedimentation, their occurrence does not necessarily imply deep water.

If the second hypothesis is correct, 100 m.y. ago the eastern segment of the Walvis Ridge would have been situated in an elevated position. Subsidence would have occurred probably by the late Cenomanian as seems to have been the case for several basins of the continental margin of West Africa¹⁸. The total subsidence is estimated to be about 2.5 km. The rate of subsidence is 25 m every million years, which is in good agreement with rates estimated by Fox *et al.*¹⁹ If our calculations are correct, this rate is similar to that of a portion of cooling lithosphere of similar age and situated at the same distance from a mid-oceanic ridge²⁰.

The results presented here suggest that the subsidence of the eastern segment of the Walvis Ridge occurred after Albian-Cenomanian time (100 m.y.BP).

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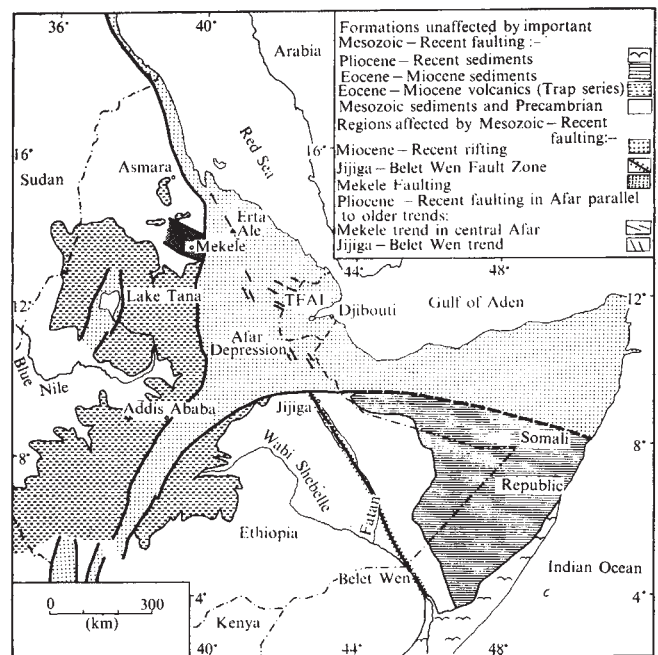


FIG. 1 The geology and large scale structure of the area around the Afar Depression.

In the Mekele area of northern Ethiopia WNW-trending normal faulting has downthrown a region about 80 km across, preserving Jurassic Antalo Limestones between uplifted blocks of Precambrian and Palaeozoic rocks. The northern margin of this region is defined by the Wukro Fault Belt which has a total throw of up to about 2 km. To the south the limestone outcrop is bounded by the Felaga Mariam Fault Belt and a cover of unconformable Lower Tertiary Trap Series basalts. Most of the movement on these faults preceded the deposition of the Cretaceous Amba Aradam Sandstone and the Trap Series, which only show minor displacements³. We consider that these faults define a graben tectonically related to the late Mesozoic tectonic movements in northern Somalia⁴.

Photographs from the ERTS satellite, and large scale topographic maps, show prominent lineaments running from the southern escarpment of the Afar Depression west of Jijiga, south-southeastwards past Belet Wen, towards the Indian Ocean north-east of Mogadisho, forming a continuation of the line of the Red Sea across the Horn of Africa⁵. Topographically the lineaments are marked by straight river courses (Gerar, Faffan and a part of the Webi Shebelle) or linear ranges of hills (for example, the Marda Range, west of Jijiga). In the latter area two parallel SSE trending crush zones about 5 km apart have been mapped. Although apparent vertical movements across these crush zones rarely exceed 100 m they both measure up to about 200 m in width and include blocks of the Mesozoic country rocks up to 100 m across which have been rotated into vertical or near vertical attitudes. These features and the overall straightness of the fault zones indicate large scale transcurent movements. Further detailed work is needed to elucidate the amount and sense of movement.

The faults affect at least the lower part of the Trap Series basalts, which are considered to be mainly of Oligocene-Miocene age in southern Ethiopia⁶. The intercalation of the lowermost basalt flows with Cretaceous(?) sandstones near Jijiga⁶ does, however, raise difficulties in the assignation of a precise lower age limit for the faulting. A further consideration here is that the main period of faulting could have preceded the basalts. An upper age limit for the faulting is obtained from the observation that they are cut by the

Early structures around the Afar triple junction

ALTHOUGH the main development of the Red Sea, Gulf of Aden and Ethiopian Rifts seems to have occurred since the Miocene^{1,2}, important earlier structures exist which seem to have determined the detailed location and trends of the later structure.

ENE-trending faults of the southern margin of the Afar Depression, which are of Lower—Mid Pliocene age⁷.

Tectonic conditions in the Afro-Arabian region have changed considerably with time. In the case of the Mekele-Northern Somalia movements there is a long time gap before later tectonic activity and we follow Sowerbutts⁸ by suggesting that this is a graben structure related to a Cretaceous Gondwana plate separation (India-Africa?), and that it is not merely an early phase of the present Afro-Arabian movements.

The tectonic relationships of the Jijiga-Belet Wen faulting are more problematical. Movement was possibly dextral, accommodating early plate separation across the Gulf of Aden, unaccompanied by Red Sea movements. This view is in direct contrast to that of Burek⁹ who suggested that such early opening of the Gulf of Aden was accommodated by sinistral transcurrent movement along the present Red Sea, and considered the Harar-Somali plateau to be a single rigid unit. The Gulf of Suez is a key area here. If the transcurrent faulting postulated by Youssef¹⁰ and others is substantiated, our work now suggests a third and more exciting possibility: an independent lower-Mid Tertiary transcurrent movement along the entire Suez-Red Sea-Jijiga-Belet Wen line which determined the locus of later plate separation. In Afar the 'oceanic' Erta'Ale volcanic range¹¹ is perfectly aligned on the Jijiga-Belet Wen structure.

It is also clear that planes of weakness along these structures, and the Mekele faulting, have locally controlled the detailed trend of later faulting within Afar. NNW-SSE (Red Sea trend) faulting occurred in the Aisha area north and north-northwest of Jijiga both before and after extrusion of the post-rifting Pliocene flood basalts¹² although this trend of faulting is uncommon elsewhere in southern Afar. Similarly WNW 'Mekele trend' faulting is seen in both pre-Tertiary rocks and in Pliocene flood basalt terrains in the southern Danakil Alps and in the Assab-Tendaho region¹³, where diamond-shaped interference patterns occur between WNW and NNW (Red Sea trend) faulting. The reappearance of these fault trends cutting Pliocene flood basalts lends further support to the view that substantial regions of the Afar are underlain by attenuated continental crust.

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Tin mineralisation above mantle hot spots

MANY important tin belts of Phanerozoic age, including those in Bolivia, Mexico, Alaska, Malaysia-Indonesia, eastern Australia and western Europe, are believed to have formed above subduction zones at convergent plate margins^{1,2}. If the late Tertiary mineralisation in the southern part of the Eastern Cordillera of Bolivia is representative of this type, then the mineralisation occurred above the deepest parts of subduction zones with the tin perhaps related to partial fusion of subducted oceanic lithosphere¹.

In addition to tin deposits at active or inactive convergent plate margins, and Precambrian tin-bearing pegmatites (for example, Congo and Western Australia), there is a third genetic type of economically important tin deposit, which is discussed here. This type formed away from lithospheric plate boundaries, in intraplate environments, and is represented by major deposits on the Jos Plateau in Nigeria^{3,4} and in Rondônia and adjoining states of southern Amazonia, Brazil^{5,6}. Smaller deposits occur in the Air and Zinder areas of Niger⁷, at Mayo Darlé in Cameroun⁸, in the Hoggar massif of southern Algeria⁹ and its extension into the Adrar des Iforas in Mali, at Sabaloka in northern Sudan¹⁰, in south-eastern Egypt¹¹, in the Tibesti area of Chad, in southern Morocco, and in the Damaraland province of South-west Africa^{11,12} (Fig. 1). Tin deposits in Transbaikalia and Mongolia are also of this type (N. Varlamoff, personal communication, 1973).

All of these deposits are associated with small, alkaline and peralkaline granite plutons of anorogenic character—the Younger Granites of Africa—that cut discordantly through the basement which is commonly of Precambrian age. The important Nigerian^{3,4} and Rondônia^{5,6} examples are typical and occur in circular to oval volcanic-plutonic complexes 5 to 20 km wide. These are characterised by early rhyolitic volcanics that are succeeded by multiple granitic intrusives, the emplacement of which was commonly controlled by ring fracturing and cauldron subsidence. Basic and intermediate rocks are also present at some centres. The granites and their porphyritic equivalents are rich in fluorine, containing accessory fluorite and topaz. Mineralisation is in the form of cassiterite accompanied by topaz, lithia mica and some wolframite in greisens and swarms of quartz veins in the roof zones of biotite granites. The complexes in certain areas, such as Hoggar¹³ and Transbaikalia-Mongolia, lack ring structures and may represent a deeper level of emplacement. Although mineralogically similar, the tin deposits in South-west Africa are somewhat different since many occur in pegmatites; at one locality, however, a stockwork of quartz veins is mined^{11,12}.

The tin-bearing, subvolcanic complexes occur in variously oriented lines, several of which constitute elongated belts, approximately 300 to 400 km long, in Nigeria, Rondônia, South West Africa (Fig. 2) and elsewhere. Progressive age trends along the lines of complexes have been claimed^{14,15} but are not strongly supported by the available radiometric data^{16,17} (Fig. 2).

Radiometric dating has demonstrated emplacement of the stanniferous complexes over a protracted time interval: late Precambrian (around 1,000 m.y. ago) in Rondônia^{18,19}, Cambrian in the Hoggar⁹ and at Sabaloka in Sudan¹⁰, Carboniferous in Air²⁰, Jurassic in Nigeria^{21,15}, Jurassic and Cretaceous in South West Africa²², Cretaceous in Trans-

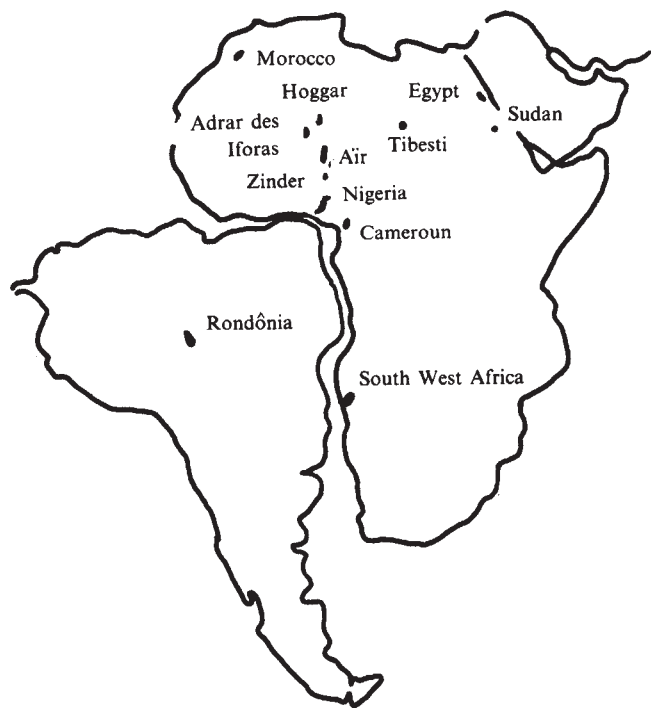


FIG. 1 Areas of tin-bearing, alkaline granitic complexes plotted on a pre-drift reconstruction of Africa and South America. The tin-bearing complex in Cameroun was, however, emplaced after the inception of spreading in the South Atlantic in the early Cretaceous³⁴.

baikalia-Mongolia (N. Varlamoff, personal communication, 1973), and Eocene at Mayo Darlé in Cameroun⁸.

The concept of hot spots or mantle plumes was introduced by Morgan^{23,24} after the earlier ideas of Wilson²⁵. Hot spots are considered to be manifestations of solid-state convection in the lower mantle, and consist of vertical columns or plumes of ascending primitive mantle material. Within the asthenosphere the plumes spread laterally^{23,24}, producing, in some cases, rifting and consequent separation of plates of continental lithosphere²⁶. The fixed mantle plumes are considered to 'burn' through lithospheric plates, thus giving rise to localised igneous activity. At the outset, when the lithosphere is continental, this activity is represented by alkaline magmatism including alkaline granitic, subvolcanic complexes^{14,15,26}, whereas once oceanic crust has been generated the plumes give rise to basaltic oceanic island chains or to aseismic ridges^{23,24,25}.

I therefore propose that the tin-bearing, alkaline granitic complexes in Nigeria, Rondônia and elsewhere were generated above mantle hot spots during a number of brief intervals since the late Precambrian. In all cases the effect of the hot spots seems to have been limited to domal uplift and the emplacement of linear belts of complexes, and activity apparently ceased before the inception of the rifting stage proposed by Burke and Dewey²⁶. In many cases the plumes sought out fundamental breaks in the continental lithosphere and these now seem to control the location and linear array of the stanniferous complexes^{16,17,20,27}. The extents of the tin-bearing belts (Fig. 2) are presumed to reflect the dimensions of the hot spots that produced them and not to indicate widespread migration of magmatism resulting from differential motion between the plumes and the overlying lithosphere^{14,15}.

Economic concentrations of tin are traditionally thought to have been derived from the continental crust (see ref. 28). The concept of tin mineralisation above rising plumes of mantle material suggests, however, that the mantle may be a possible source for the tin and accompanying tungsten and fluorine. A source in the mantle is difficult to prove be-

cause extraction from continental lithosphere, by magmas or magmatic heat provided by rising plume material, cannot be easily discounted. Initial Sr⁸⁷/Sr⁸⁶ ratios of 0.721 and 0.718 determined for samples of granites from complexes in Nigeria²⁹ and Rondônia¹⁸ suggest that significant amounts of the granitic rocks associated with the tin deposits have a crustal source, a hypothesis also supported by other workers²⁶. Much lower initial Sr isotope ratios have, however, been obtained for some granitic units of the White Mountain plume trace in New Hampshire³⁰, possibly indicating a subcrustal origin for their parent magmas. In Nigeria, tin potentially available for extraction, occurs in pegmatites in the Precambrian basement³¹. The abundance of boron, beryllium and tantalum in these deposits and the virtual absence of these elements from the Jurassic tin deposits have been cited as evidence against the hypothesis of remobilisation of Precambrian tin²⁷, unless some mechanism for element segregation is proposed. The rather widespread occurrence of tin in association with anorogenic, alkaline granitic complexes and, perhaps more significantly, its presence along the full length of belts of complexes, also tend to decrease the likelihood of a source in remobilised Precambrian deposits. Furthermore, the absence of significant amounts of the more common base metals from these complexes seems to preclude anatexis or assimilation of unmineralised Precambrian crustal rocks as an origin for the tin. Although some of the granitic rocks are derived at least in part from the continental crust, the evidence, although far from unequivocal, seems to favour a source in the mantle for the tin (compare with ref. 27).

Support for a mantle derivation of lithophile elements at hot spots is provided by evidence from two localities at which continental crust is absent. Blocks dredged from the axial trough of the mid-Atlantic Ridge in the vicinity of the Azores hot spot show evidence of hydrothermal attack and have high contents of several heavy metals, including tin³². At the Iceland hot spot, granitic rocks derived from parent magmas of mantle origin contain concentrations of molybdenum³³, an element which has a geochemical affinity to tin and which occurs with it in some of the complexes (for example, Nigeria⁴ and Sudan¹⁰).

The generation of tin mineralisation above some hot spots and not above others (White Mountain magma series, western Scotland Tertiary province) might reflect either an inhomogeneous distribution of tin (or fluorine) in the mantle or

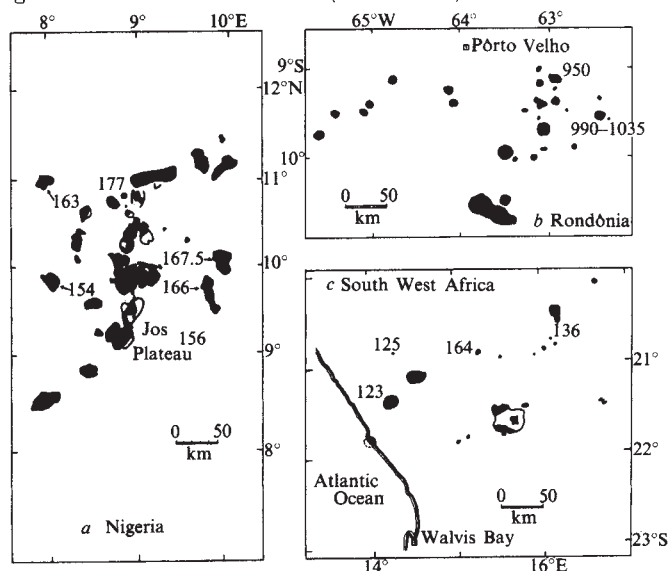


FIG. 2 Linear distribution of tin-bearing, alkaline granitic complexes within elongate belts in a, Nigeria; b, Rondônia; and c, South West Africa. Radiometric ages (numbers) in Nigeria from Jacobson *et al.*²¹ and van Breemen and Bowden¹⁵; in Rondônia from Priem *et al.*¹⁸; and in South West Africa from Siedner and Miller²².

the propagation of mantle plumes, each with a different tin (or fluorine) content, from several levels in the mantle.

The concept of tin mineralisation related to granitic complexes of alkaline type generated in intraplate environments, and unrelated to arc-trench systems, should be useful in the search for ore. In poorly exposed regions, such as Rondônia, the observation that hot-spot activity commonly gives rise to several lines of complexes within an elongated belt (Fig. 2) could facilitate the delimitation of areas for more detailed exploration. Tin deposits might also accompany felsic differentiates at some hot spots in the ocean basins.

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Nature of South Africa's Cape Fold Belt

THE report by Gough¹ of a probable ridge on the highly conductive zone of the upper mantle may be of considerable significance in understanding the deformation of the Palaeozoic trough which became the Cape Fold Belt of South Africa. Until the results of the extended magnetic survey are available, detailed interpretation would be premature, but a few comments may offer guidance for future geophysical work.

The Cape Fold Belt is difficult to explain on plate tectonic models, since (a) it shows a complete lack of any significant igneous or regional metamorphic activity, and (b) it consists of two folded chains almost at right angles, the main one trending E-W, and the smaller one at its western end NNW. These two belts meet at what can best be described as a convergence, since both swing round to a SW direction and merge (Fig. 1).

I have suggested² that an explanation should rather be sought in terms of vertical tectonics and gravity sliding, and a model based on this premise was worked out. The immediately relevant part of this model involved uplift of the southern flank of the main depositional trough (whose axis of maximum subsidence runs just north of the present-day mountain belt) to provide the slope on which gravity sliding took place. It seemed necessary, however, to explain certain zones of more intense folding which occur just north of the major E-W faults, and it was suggested that these faults were active at a very early stage, and with downthrows to the north, forming a series of steps down which gravity sliding took place.

At a later stage, during the breakup of this part of Gondwanaland, the prevailing crustal tension would have induced new movement along these existing lines of weakness but this time with downthrows to the south, as seen today.

The conductive anomaly as shown in Gough's Fig. 1 underlies an area of maximum uplift indicated by the outcropping of late Precambrian basement, on the north side of a major fault line, and it is therefore important to know whether the anomaly is confined to the vicinity of the fault, or whether it extends much further southward (the latter is implied by Gough's statement (ref. 1, page 94) that less than half the anomaly was covered by the array of magnetic stations and by Fig. 9 in ref 3). It is also important to know whether the same or similar anomalies are associated with other fault lines further south.

Gough suggests that the conductive anomaly may pass eastwards into the gravity anomaly described by Hales and Gough⁴, which is associated with the Cretaceous-filled Algoa basin on the depressed southern side of one of the major

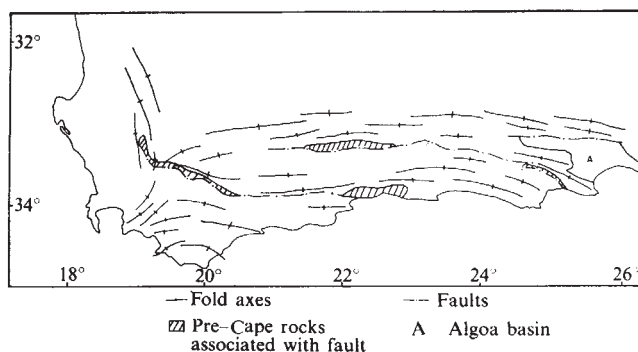


FIG. 1 The Cape Fold Belt.

faults. If, however, there is any close connection between the proposed upward bulge (I prefer this term to 'plume' in this context) on the surface of the highly conductive mantle and the crustal uplift, the conductive anomaly should pass north of the gravity anomaly; it will be interesting to see what the extension of the magnetic survey reveals.

The basic mechanism I invoked for the formation of the Cape Fold Belt was one of vertical movements of fault-defined blocks but no driving force for such movements could be suggested at that time. Clearly, however, if Gough's interpretation of the conductive anomaly is correct, and if such linear mantle bulges can form and decay over long periods, they could provide an adequate explanation for 'keyboard tectonics' in the overlying crust and thus may explain not only the recent uplift of the Cape mountains but also their earlier generative stages.

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Oxidation of natural olivines

THE oxidation of olivine is a commonly recognised natural phenomenon and has been investigated experimentally using crystallographic and thermodynamic techniques^{1,2}. It is generally believed that the oxidation of the olivine occurs by initial breakdown of the fayalite component, and subsequent reaction with the forsterite component, to give magnetite and orthopyroxene³. The oxidation of olivine of composition Fo₈₀ can be envisaged as two part reactions:



that is,



In natural olivines such a reaction is commonly represented by partial or complete replacement of the olivine by orthopyroxene-magnetite aggregates. A variation of this reaction is, however, observed in several suites of Precambrian olivine-bearing cumulate rocks which are intrusive into granulite facies gneisses in the western Musgrave Block in central Australia. In such cases a compositional control on the oxidation process is apparent.

(1) In relatively fayalitic olivines (Fo₈₃₋₇₅), olivine is partially or completely altered to granular orthopyroxene-vermicular magnetite aggregates.

(2) In more forsteritic olivines (Fo₇₈₋₈₄), primary spinel inclusions in the olivine are surrounded by thin (0.01 to 0.2 mm) rims of fibrous to granular orthopyroxene. Small quantities of vermicular to granular dark green spinel are commonly intergrown with the orthopyroxene. The spinel core is largest where associated with the thickest rims, suggesting that the primary spinel has grown by the addition

of reaction product spinel. Limited electron microprobe data suggest that both spinel types are of similar picotite composition. Spinel inclusions in other phases are not rimmed.

(3) In the most forsteritic olivines (Fo₈₅₋₉₀), no signs of alteration were observed even though euhedral green spinel inclusions were present.

It is believed that the orthopyroxene-magnetite intergrowths in the more fayalitic olivines result from subsolidus oxidation by the type of reaction shown for olivine of composition Fo₈₀.

Such a reaction involves a volume decrease of approximately 3%. Relative volumes of one magnetite to three enstatite would be produced and this is approximately what is observed. The orthopyroxene produced would be much more Mg-rich than the primary olivine as a result of the oxidation of the fayalite molecule. Both optical and electron microprobe examinations support this prediction.

Granular orthopyroxene and opaques, apparently pseudomorphous after olivine, have been reported in granulite facies rocks apparently penetrated by a transient oxidising fluid phase⁴. Very large areas of the Adirondacks granulite facies terrain have undergone oxygen isotope exchange with some pervasive fluids⁵. The cause of oxidation in the central Australian rocks is, however, unknown. The oxidation may be related to the development of interstitial hydrous fluids, because olivines of composition Fo₈₃ in the Kalka Intrusion are only oxidised in rocks containing biotite. Alternatively, reheating during post-consolidation deformation in the area may have increased oxygen fugacity⁶.

The oxidation is relatively late stage. Coronas typical of the subsolidus high pressure olivine-plagioclase reaction (olivine + plagioclase → orthopyroxene + clinopyroxene + spinel) are commonly observed in this area. In some cases these double coronas surround orthopyroxene-opaque aggregates, indicating that oxidation occurred after the olivine-plagioclase reaction.

The orthopyroxene-spinel intergrowths presumably formed by similar oxidation of more magnesian olivines in the presence of spinel:



The spinel is picotite, (Fe, Mg) (Al, Fe, Cr)₂O₄, which corresponds to the magnetite, FeFe₂O₄, in the more iron-rich oxidation reaction. The presence of chromium in at least some olivine lattices is indicated by the chromite⁷ or chrome magnetite exsolution plates in some of the olivines from the area. The chromium was probably originally incorporated into the olivine as Cr²⁺ in isomorphous substitution with Mg²⁺ and Fe²⁺ (ref. 8). Part of the equivalent oxidation reaction for more magnesian olivines could therefore be:



that is, the spinel phase would contain some chromite component.

The origin of the aluminium in the spinel is more difficult to explain. It may have been derived from the associated primary spinel inclusions (as could the chromium) which were subsequently homogenised with respect to the vermicular spinel. The alternative source, Al-bearing phases (for example plagioclase) outside the olivine, involves diffusion problems because movement would need to be through the olivine lattice. It is, however, interesting to note that Muir and Tilley⁹ propose movement of Mg, Fe and Si through olivine lattices during similar oxidation processes in some Kilauean picrite basalts.

The lack of oxidation in the most forsteritic olivines, even in the presence of primary spinel inclusions, indicates the greater stability of the more magnesian olivines under oxidising conditions.

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Stream abrasion of flint implements

Most finds of European Lower Palaeolithic flint artefacts are made from ancient river gravels, either as what are assumed to be concentrations of freshly made implements, suggesting an occupation site, or as abraded material indicating stream redeposition. Verbal description of any abrasion is inaccurate, because lightly abraded specimens may appear fresh to the naked eye. The widths of ridges can be measured on implements with a microscope, thus allowing unworn artefacts and distinct assemblages within a given population to be identified.

When flakes are struck off a flint nodule, either by natural or human agency, the resulting flake scars are separated by a narrow ridge, which is extremely sharp in the fresh state. As the nodule becomes abraded the width of the ridges increases and is easily measurable. The abrasion rate is proportional to the relative hardness of the flint, the velocity of the current, the shape of the nodule or implement, and the particle size distribution of the material present in the transporting medium.

Ridge widths can be measured by placing the artefact under a microscope with incident light from two separate sources converging on the ridge. At least 25 ridges from each

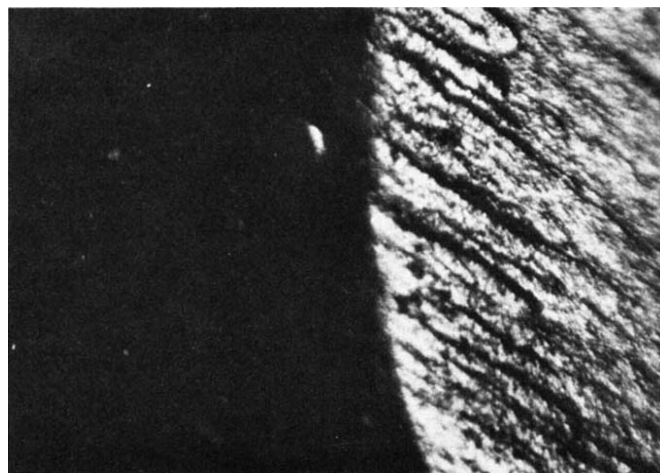


FIG. 2 The appearance of stress cracks. Average width 15 μm .

side of the implement must be measured. Care should be taken to avoid those that have been affected by human use. A suitably calibrated eyepiece and a magnification of $\times 75$ should permit accuracy of $\pm 0.1 \mu\text{m}$. Various chemical coatings, for example silver nitrate, may be used to improve the optical properties of flint for photography, but these tend to obscure the ridges. Direct comparison of the ridge widths of the components of an assemblage is then possible, and may easily distinguish unworn material.

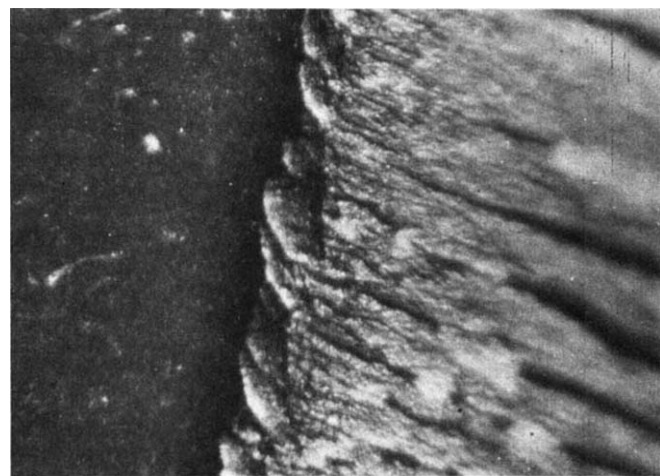


FIG. 3 A 'braided' ridge. Average width 53 μm .

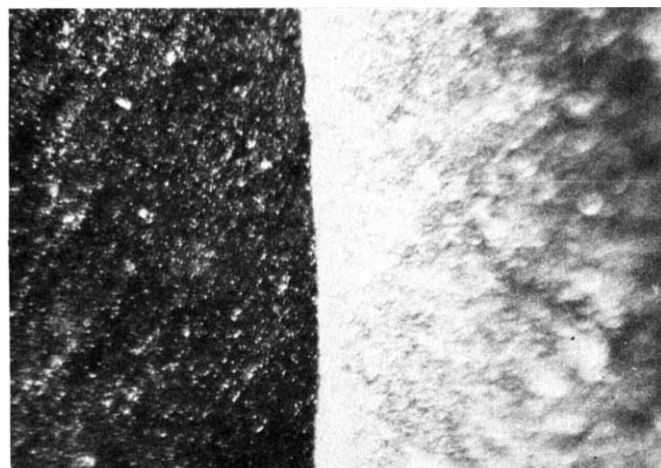


FIG. 1 The fresh ridge. Average width 5 μm . Magnifications of Figs 1-6, $\times 75$.

Figures 1-6 show stages in the natural abrasion of a ridge. They illustrate the process and act as standards for the terminology employed. Figure 1 shows a fresh ridge photographed at a magnification of $\times 75$ in incident light. The ridges have average widths of $5 \mu\text{m}$. The abrasion process consists of both chipping of the implement by other natural and humanly worked nodules during transport, and of a slow grinding down of the ridge by an abrasive consisting of the finer elements in the load carried by the stream. The chipping process is much more active soon after the beginning of the abrasion but continues throughout the process at the places nearest to the centre of gravity of the nodule. Initial stages are marked by the appearance of stress cracks (Fig. 2) which develop a 'braided' appearance (Fig. 3). The braiding is caused by pebbles hitting the ridge at an acute angle and striking off microscopic flakes. The braided ridge is then ground away (Fig. 4), causing a gradual increase of ridge width, irrespective of the flaking angle of the ridge. An alternative sequence involves the rapid appearance of percussion craters, caused

TABLE 1 Correlation between observed ridge widths and commonly used verbal descriptions

Observed ridge width (μm)	0-10	10-20	20-50	50-100	100-200	200-300	300+
Common verbal description	Mint condition	Very fresh	Fresh	Slight abrasion	Abraded	Heavily abraded	Very heavily abraded
Suggested index value	0	1	2	3	4	5	6

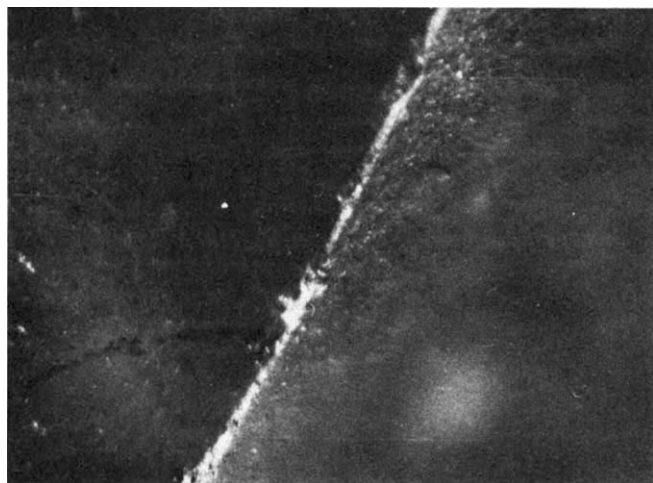


FIG. 4 A 'braided' ridge subjected to grinding in fine clay. Average width $34 \mu\text{m}$.

by the impact of a heavy pebble (Fig. 5). The final product is, however, always a smooth ridge crossed by hairline cracks (Fig. 6). The worn ridge may attain a width of 4 mm in heavily abraded material. The nature of the abrasion process is largely a function of the type of abrasive and it is possible to distinguish implements that have been abraded in different environments. If the abrasive is fine grained, such as silt or clay, the relative abrasion is very slow but the rounded appearance of the ridge (Fig. 6) is attained very quickly. Experimental verification of these processes may be obtained by abraiding freshly made implements in a tumbling mill, or sandblaster, with different abrasives and concentrations.

Table 1 shows a correlation between the observed ridge widths and the commonly used verbal description of abra-

sion. An 'abrasion index' value is suggested, based on these metric criteria, facilitating easy quantification of results.

Using these methods it may be possible to define the depositional environment of stray artefact finds by commenting on the textural character of the abrasive material present in the stream, and to distinguish groupings within apparently homogeneous artefact population. In several cases doubt has been cast on supposedly *in situ* assemblages, which now appear to have been stream rolled. I have recently examined a late Lower Palaeolithic assemblage from river gravels at Great Pan Farm (Newport, Isle of Wight). It was at first

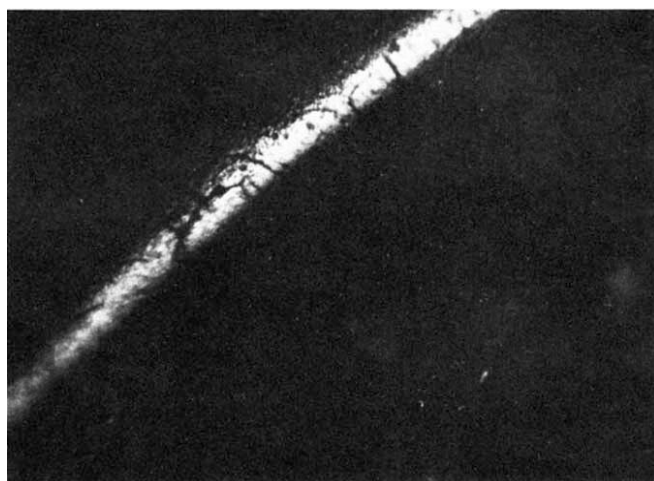


FIG. 6 Heavily abraded ridge showing the final rounded appearance, crossed by fine hairlike cracks. Average width $279 \mu\text{m}$.

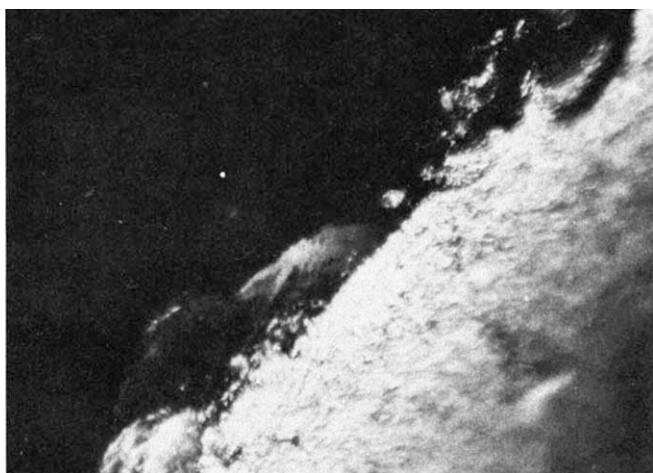


FIG. 5 Ridge showing some grinding and percussion craters formed by the impact of large pebbles. Average width $81 \mu\text{m}$.

thought that the implements were manufactured at a nearby occupation site, implying that they were virtually contemporary with the gravel deposit, because they appeared totally unabraded. Closer examination showed that many exhibited clear traces of stream abrasion, and variable abrasion index values. It was therefore concluded that the material represented a random accumulation produced by selective stream redeposition, necessitating a re-examination of the typological characteristics of the false 'assemblage'.

A study of the standard typological sequences using these methods may emphasise the need for caution in interpreting implement associations.

I thank Mr A. M. ApSimon and Dr D. P. S. Peacock for help and criticism. The idea of obtaining experimental verification of the abrasion process was developed on the basis of suggestions and practical help from Peacock.

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BIOLOGICAL SCIENCES

Virus productive transformation of marsupial cells by Schmidt-Ruppin strain of RSV

Rous sarcoma virus (RSV) transforms different species of mammalian cells. The RSV genome is usually not fully expressed in such cells and the virogenic type of virus-cell interaction is obtained^{1,2}. One exception was, however, noted by Svoboda and Klement³ in the case of some hamster tumours induced by Prague strain of RSV (PR RSV) which were found to produce traces (about 1 ID₅₀ per 2.5 g tumour tissue) of infectious RSV.

In order to establish a suitable RSV-producing system in mammalian cells which would be well defined karyologically, we used the marsupial cell line PtK 1 originally derived from the kidney of rat-kangaroo (*Potorous tridactylis apicalis*) by Walen and Brown⁴.

Cells were cultured in medium 199 supplemented with 20% foetal bovine serum. For the transformation, 1.5×10^6 PtK 1 cells were mixed either with 6.4×10^8 resistance-inducing factor (RIF)-free Brown Leghorn chicken fibroblasts (BLEF) infected with Schmidt-Ruppin strain of RSV (SR RSV), or with the same amount of SR RSV-infected BLEF cells which were irradiated with 7,100 R. The mixed cell cultures were designated RSPtK 1 and RS(X)PtK 1, respectively. SR RSV used (SR RSV K18) represents the pure subgroup D virus isolated from a single focus which appeared in the mixed culture of virogenic mammalian cells and chicken fibroblasts⁵. Mixed cultures as well as control PtK 1 cells were subcultured in Roux bottles and split in a ratio of 1:3 at intervals of 3–10 d.

The clone RSPtK 1/K1 was derived from RSPtK 1 cells after 3 month cultivation by picking a single colony of cells containing refractile cells. For colony isolation, a stainless steel cylinder fixed with a silicone grease to the bottom of the dish was used. The plating efficiency of RSPtK 1 cells was 1%. RSPtK 1/K3 clone was isolated by seeding one cell with a micropipette on the feeder layer of X-irradiated (7,000 R) PtK 1 cells prepared on Petri dishes. The growing clone was obtained in one out of ten dishes.

After 4 month cultivation the cell lines were checked for the presence of avian gs antigen using the immunofluorescence method. This antigen was detected in both RSPtK 1 and RS(X)PtK 1 cells.

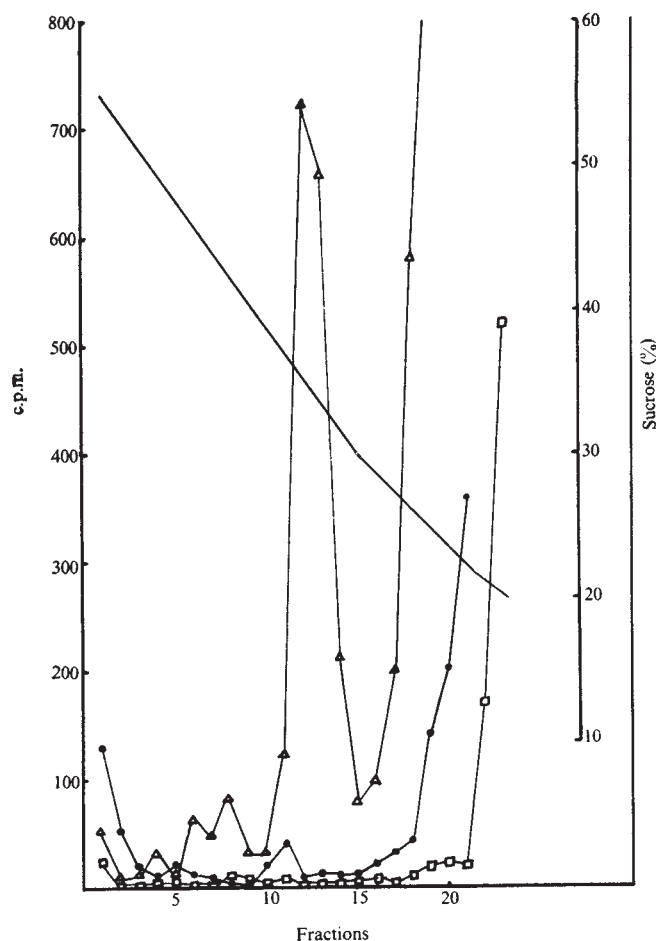


FIG. 1 Culture medium (10 ml) from virus producing RSPtK 1 and control PtK 1 cells labelled for 24 h with $10 \mu\text{Ci ml}^{-1}$ of ^3H -uridine or ^3H -thymidine was clarified by centrifugation at 10,000 r.p.m. for 10 min and layered over 50 ml 10%–58% linear sucrose gradient. The samples were centrifuged in the MSE 3×65 ml rotor in a MSE 65 MkII ultracentrifuge at 20,000 r.p.m. at 6°C for 4 h. Fractions of 2 ml were collected from the bottom and assayed for sucrose concentration and after TCA precipitation for radioactivity. The maximum radioactivity of incorporated ^3H -uridine was found in the peak of density 1.156 which is characteristic of C-type RNA viruses. No radioactivity was found after ^3H -thymidine labelling, as well as in the control cells. Δ — Δ — Δ , ^3H -uridine, RSPtK 1; $\circ$ — $\circ$ — $\circ$, ^3H -uridine, PtK 1; $\square$ — $\square$ — $\square$, ^3H -thymidine RSPtK 1.

In addition, all cell lines were tested for the presence of RSV genome and infectious RSV. Living cells from RSPtK

TABLE 1 History and biological properties of PtK 1 cells and RSV-transformed cell lines derived from them

Designation of the cell line	Mode of transformation	Morphology	Generation time (h)	Plating efficiency in soft* agar	Modal chromosome No.	Virus production (FFU ml ⁻¹)
PtK 1	—	Normal	45	$<10^{-6}$	11	0
RS(X)PtK 1	Co-cultivation with chicken fibroblasts infected with SR RSV, irradiated with 7,000 R	Normal to transformed	34.5	$<10^{-6}$	11	12
RSPtK 1	Co-cultivation with chicken fibroblasts infected with SR RSV, without X-irradiation	Transformed	20.5	10^{-3}	11	22 25 (with 100 μg DEAE dextran) 15 (filtrate) 50
RSPtK 1/K1	Clone derived from RSPtK 1 cells	Transformed	NT	10^{-5}	11	
RSPtK 1/K3	Clone derived from RSPtK 1 cells	Transformed	NT	NT	11	2

* Average number from two dishes



FIG. 2 Morphology of RSPtK 1 cells which shows a non-oriented pattern of growth and presence of stellate and fibroblastoid cells ($\times 55$).

1, RS(X)PtK 1 and RSPtK 1/K1 cells gave rise to Rous sarcomas after injection of relatively small numbers of living cells (about 10^3). Moreover, infectious virus was detected when chickens were injected with cell-free culture fluid collected from full-grown cell layers, either centrifuged at 3,000g for 30 min or filtered through Millipore membranes (0.22 μ m pore size).

The mean titre of virus as measured by the focus assay method⁶ varied from 1.2×10^4 to 5×10^4 FFU ml⁻¹ between different lines with the exception of RSPtK 1/K3 clone kept for a long period of time in tissue culture where RSV production was lower (Table 1).

RSPtK 1 cells were also studied by electron microscopy. Cells were fixed with glutaraldehyde, scraped with a rubber policeman, pelleted, post-fixed with osmium tetroxide and after dehydration the pellets were embedded in Araldite. Ultrathin sections were cut with a Reichert Om U 2 ultramicrotome, contrasted with uranyl acetate and lead citrate and observed with a JEM 7 electron microscope. Early stages of budding of virus particles as well as mature C-type particles were clearly identified.

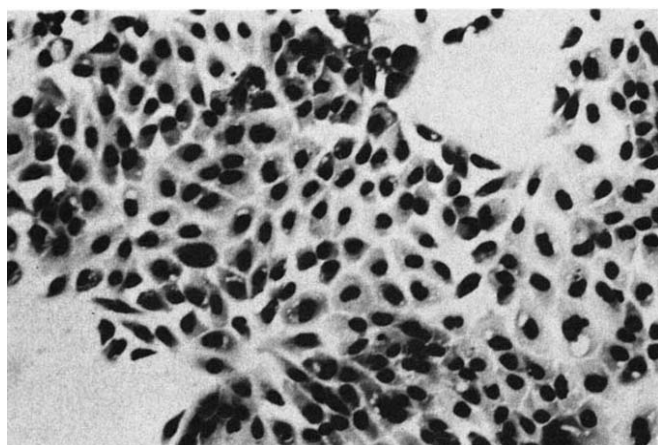


FIG. 3 Morphology of PtK 1 cells. Epithelioid cells with the tendency to form packed colonies ($\times 55$).

If RSPtK 1 cells were grown in the medium containing ³H-uridine and the supernatant was centrifuged in 10–58% sucrose density gradients a peak of radioactivity at a density of 1.16 g cm⁻³ was detected (Fig. 1). The virus isolated from RSPtK 1/K1 cultures was neutralised by two log dilutions with the specific anti-SR RSV-D antiserum⁵ to the same extent as SR RSV-D virus. Transformed cell lines cultivated

over a year tended to lose their virus-producing capacity and to become virogenic.

Further biological parameters of all cell lines are summarised in Table 1. RSPtK 1 and RSPtK 1/K1 cell lines plated in soft agar⁷ and were morphologically transformed. Figure 2 illustrates the morphology of RSPtK 1 cells which displayed a non-oriented pattern of growth; the cell population consisted of stellate and fibroblastoid cells. The generation time of RSPtK 1 cells was half that of control PtK 1 cells. In contrast to it, PtK 1 and RS(X)PtK 1 cell lines did not form colonies in soft agar and retained the epithelioid morphology with a tendency to form packed colonies on the solid substrate (Fig. 3). But after prolonged passages RS(X)PtK 1 cells also became morphologically transformed.

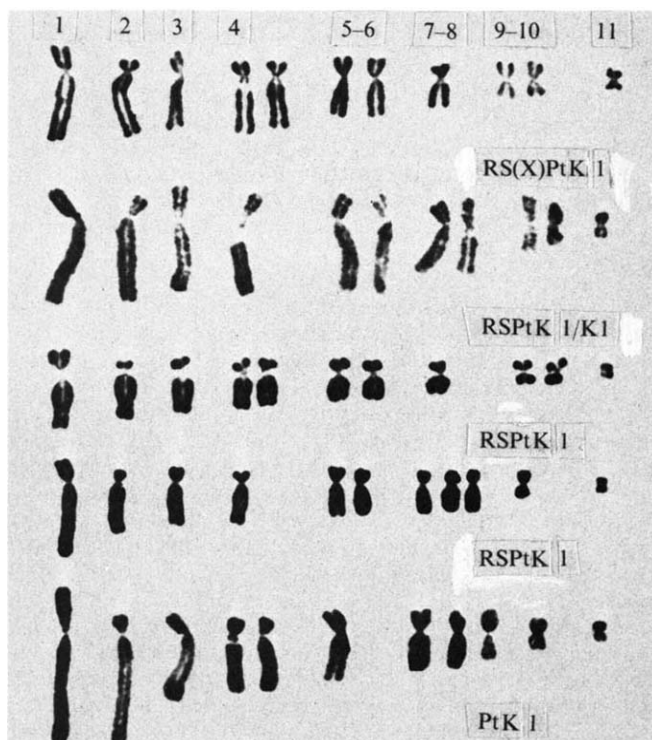


FIG. 4 Karyotypes of PtK 1 cells and RSV-producing cell lines derived from them ($\times 544$).

All cell lines had the eleven chromosome stemlines (Fig. 4) and the frequency of chromosomal damage was not increased above the control background in the RSV-producing lines.

The model of RSV-transformed PtK 1 cells offers a unique possibility for investigating RSV production on a permanent cell line which is well defined karyologically and for studying which host cell chromosomes are necessary for the maintenance of the virus-producing state.

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DNA synthesis in 'nuclear monolayers' from BSC-1 cells infected with herpes virus

HERPES simplex viruses (HSV) produce latent (as well as overt) infection in man. Recent evidence seems to indicate that they can also transform mammalian cells in culture^{1,2}. Furthermore, seroepidemiological³ and molecular hybridisation⁴ studies have suggested a possible causal relationship between genital infection with HSV type II and cervical cancer.

A detailed understanding of viral DNA replication is necessary to elucidate the process of HSV infection. Considerable information has been obtained about the changes, caused by infection, in the soluble enzymes associated with DNA synthesis, for example, the induction of a virus-specific DNA polymerase^{5,6} and deoxynucleoside kinase^{7,8}, soon after infection.

In recent years, however, the use of carefully isolated, insoluble replication complexes (or toluenised cells) has transformed our views on the roles of soluble polymerases in the replication of the bacterial chromosome⁹⁻¹¹. It therefore seemed likely that similar insights into the mechanism of herpes DNA replication might be obtained with an analogous mammalian system. Friedman and Mueller¹² have demonstrated the ATP-dependent incorporation of deoxynucleoside triphosphates into the DNA of isolated HeLa nuclei and showed that this activity was correlated with the synthetic capacity of the intact cells. To prepare the active nuclei the cells were disrupted in hypotonic buffer, the cytoplasm was removed by repeated centrifugation and the nuclei suspended in a suitable buffer. The main drawbacks of this

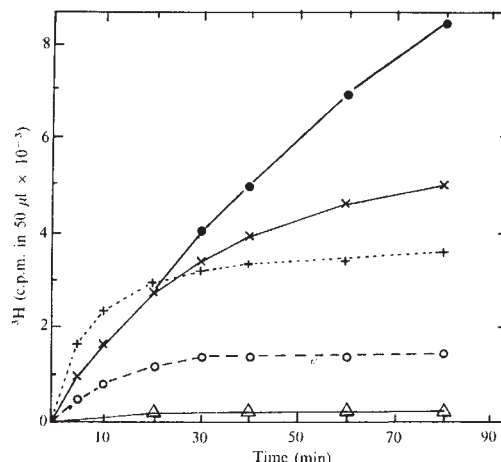


FIG. 2 Kinetics of ³H-dTTP incorporation at 37° C. BSC-1 cells were grown to confluence in Eagle's medium in 5 cm Petri dishes and infected at a multiplicity of infection of 10 with HSV type 1 strain α (2 × 10⁷ P.F.U. per 0.2 ml per dish) or mock-infected with 0.2 ml medium. After 1 h the cell sheets were washed twice with 3 ml medium and overlaid with a further 2 ml. At 6 h after infection the medium was removed and 2 ml of cold 0.5% NP40, 1 mM MgCl₂ and 0.5 mM Hepes buffer, pH 7.6 were added. After a minute or two this was decanted and the resulting 'nuclear monolayers' washed with 1.0 ml of the incorporation buffer minus ³H-dTTP (see text), at various pH values. Complete incorporation buffer (0.5 ml) at the appropriate pH was then added to each dish and the dishes incubated in sealed humidified boxes for 45 min at 37° C. The reaction was stopped by adding 2 ml ice-cold 0.15 M NaCl/0.015 M sodium citrate/0.01 M EDTA. The nuclei were scraped off, transferred to tubes, made 0.5% with respect to sodium dodecyl sulphate and heated at 65° for 10 min. After cooling, pronase (500 µg ml⁻¹) was added and the mixture incubated for 16 h at 37° C. Samples (50 µl) were taken and the acid-insoluble radioactivity determined by the paperdisk method of Bollum¹⁹. ×, pH 7.6; ●, pH 7.6 but more dNTPs (25 pmol dATP, dCTP, dGTP and 10 µCi ³H-dTTP per dish) added after 20 min; +, pH 7.9; ○, pH 7.1; Δ, nuclei from mock-infected cells, at pH 7.6.

method are that preparation takes at least 30 min, nuclei can be damaged, or lost, due to clumping or rupture during handling and the nuclear pellet is difficult to re-disperse completely.

This report describes a quick and convenient method of nuclear preparation and the results obtained by its application to the study of DNA replication in BSC-1 cells infected with wild-type (wt) and temperature sensitive (*ts*) mutants of herpes simplex viruses. A brief report of the use of similarly prepared 3T3 cell nuclei in the study of RNA transcription has recently appeared¹³.

If BSC-1 cells are treated *in situ*, with the non-ionic detergent NP40 (0.5% in 25 mM HEPES buffer pH 7.6 containing 1 mM MgCl₂, 0.5 mM NaCl, and 1 mM dithiothreitol) the cytoplasm is removed, leaving the nuclei and part of the cell membrane firmly attached to the culture vessel as a 'nuclear monolayer' (Fig. 1). Some shrinkage of the nuclei does occur but few are lost and no whole cells are detectable. The nuclear monolayer is stable and can be washed to remove cytoplasmic contamination. Autoradiographic evidence suggests, however, that some ribosomes remain attached to the residue of the cell membrane which surrounds each nucleus.

When BSC-1 cells which have been infected with herpes simplex viruses are treated in this way 5-9 h after infection (when viral DNA synthesis is most active *in vivo*) the resulting nuclei retain a high capacity for viral DNA synthesis. Under the appropriate conditions (50 mM HEPES buffer, pH 7.6, containing 10 mM MgCl₂, 100 mM NaCl, 0.5 mM CaCl₂, 1 mM dithiothreitol, 50 µM dATP, dCTP and dGTP, 20 µCi ml⁻¹ ³H-dTTP and 5 mM ATP, see

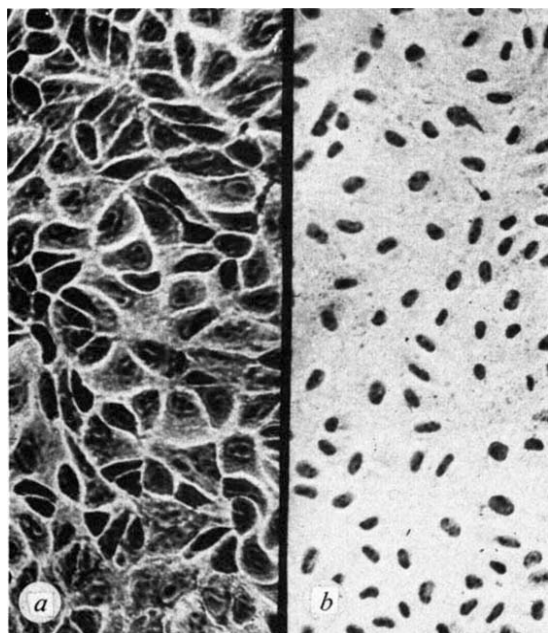


FIG. 1 A confluent monolayer of BSC-1 cells before (a) and after (b) treatment with 0.5% NP40 25 mM HEPES buffer pH 7.6, 1 mM Mg Cl₂ and 0.5 mM CaCl₂.

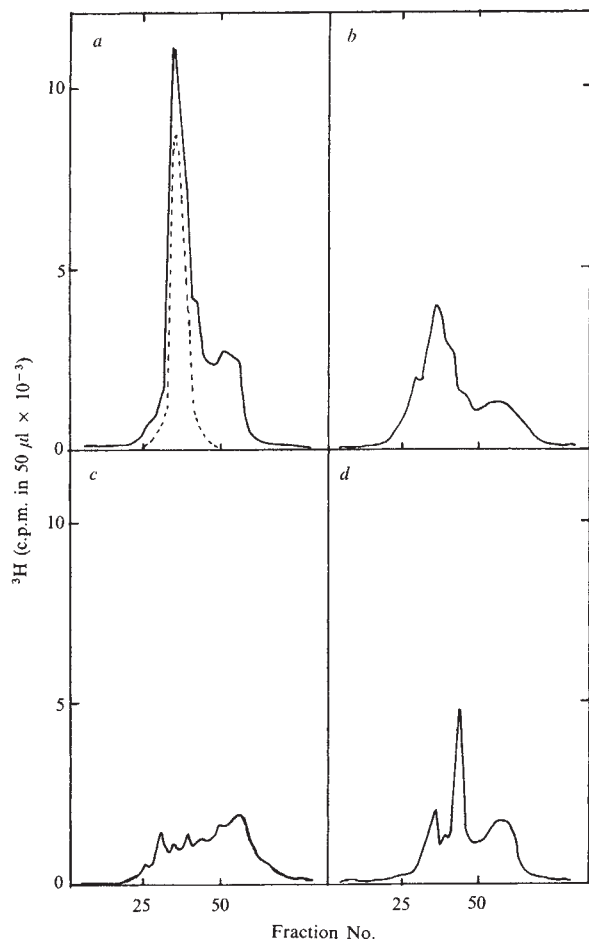


Fig. 3 Pycnographic analysis of DNA labelled *in vitro* with ^3H -dTTP in modified incorporation mixtures. *a*, Complete incorporation mixture as described in the text; *b*, complete mixture minus ATP; *c*, complete mixture minus NaCl; *d*, complete mixture but with ATP, CTP, GTP and UTP at $30\ \mu\text{M}$. Incorporation conditions and DNA analysis as described in Figs 2 and 6. The position of virion DNA (.....) is also shown in (*a*).

Fig. 2) extensive synthesis occurs. Up to 100 pmol (around 100,000 c.p.m.) can be incorporated per 10^6 nuclei in 60 min at 37°C , and the initial rate of synthesis, during the first 5 min can be as high as $10\ \text{pmol min}^{-1}$. These figures, which are minimum estimates based on the incorporation of added ^3H -dTTP (making no allowance for any endogenous dTTP) compare very favourably with recently published studies in which the techniques of Friedman and Mueller were used to examine the replication of DNA in nuclei from adenovirus KB cells infected with adenovirus type 5^{14,15} and 3T6 cells infected with polyoma¹⁶. Although incorporation falls off fairly rapidly, the addition of more deoxyribonucleoside triphosphates (dNTPs) at 20 min maintains the existing rate for almost another hour (Fig. 2), indicating that a major cause of the reduction in the rate of synthesis is probably lack of substrate; due presumably to degradation. This has not been checked directly.

The addition of more ATP at this time has no effect. This is perhaps to be expected as the ATP concentration in the mixture (5 mM) is already fairly high. Although ATP is required for full activity, a substantial, but variable, amount of incorporation (10–65% of that in the complete mixture) occurs even in its absence. It is not clear whether this is due to residual endogenous ATP or to a DNA synthetic activity which does not require ATP.

If magnesium, or all four dNTPs, are omitted, however, synthesis is reduced to less than 5%. Similarly, if NaCl is not added to the incorporation buffer, synthesis, particularly

of viral DNA (see Fig. 3), is severely inhibited. Dithiothreitol, (the omission of which has little effect) is added as a precautionary measure since the sulphhydryl antagonist N-ethylmaleimide (at 20 mM) completely inhibits DNA synthesis in these nuclear preparations.

Under these conditions nuclei prepared from mock-infected cultures exhibit almost no DNA synthesis (Fig. 2). Since these cells are subject to contact inhibition of growth and movement, and have been confluent for at least 2 d before use, there is little *in vivo* DNA synthesis compared with a logarithmically growing population. The activity detected *in vitro* is, however, considerably lower than would be expected. This may mean that the residual DNA synthesis in resting cells depends on a soluble polymerase which is lost during preparation of the nuclei.

As expected, nuclei from virus-infected cells will incorporate radioactivity from any of the four dNTPs. It is, however, surprising to find that ^3H -TdR is also readily incorporated, provided the other three dNTPs are supplied (see Fig. 4). No incorporation occurs in the absence of ATP, nor can any of the other deoxyribonucleosides be incorporated. This activity is not therefore due to a few whole cells which have survived the detergent treatment. Since these nuclei are washed before assay, it seems that this activity is bound either in, or to, the nucleus, or alternatively, to the remains of the cell membrane.

This observation is of particular interest since it is well established that one consequence of herpes virus infection is the appearance of a virus-specific thymidine kinase^{7,8} shortly before the onset of viral DNA synthesis. In mock-infected cells very little ^3H -TdR is incorporated.

A direct comparison of the absolute amounts of DNA synthesised *in vivo* and *in vitro* in a given time cannot be made without greater knowledge of the many parameters involved (such as pool sizes and rates of synthesis). It is not known whether initiation of new rounds of DNA synthesis occurs *in vitro* or whether those molecules already in the process of replication when the nuclei are prepared are merely completed. Nevertheless, when the DNA synthesised *in vitro* is banded on a CsCl gradient the relative amounts of viral and cellular DNA always reflect the *in vivo* pattern throughout the viral growth cycle (see Fig. 5). Closer examination of the CsCl profiles, however, reveals that the *in vitro* labelled viral DNA appears to consist of several species with slightly different densities, rather than a single homogeneous peak. This heterogeneity (a trace of which can often be seen even in viral DNA prepared from whole cells) is more noticeable

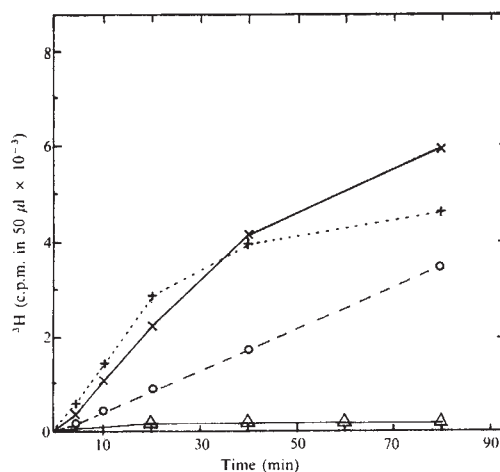


Fig. 4 Kinetics of ^3H -TdR incorporation at 37°C . Cells were infected and nuclei prepared as in Fig. 2. The incorporation protocol was also the same, except that ^3H -TdR ($20\ \mu\text{Ci ml}^{-1}$) replaced dTTP in the incorporation mixture. $\times$, pH 7.6; $\circ$, pH 7.1; $+$, pH 7.9; Δ , nuclei from mock-infected cells at pH 7.6.

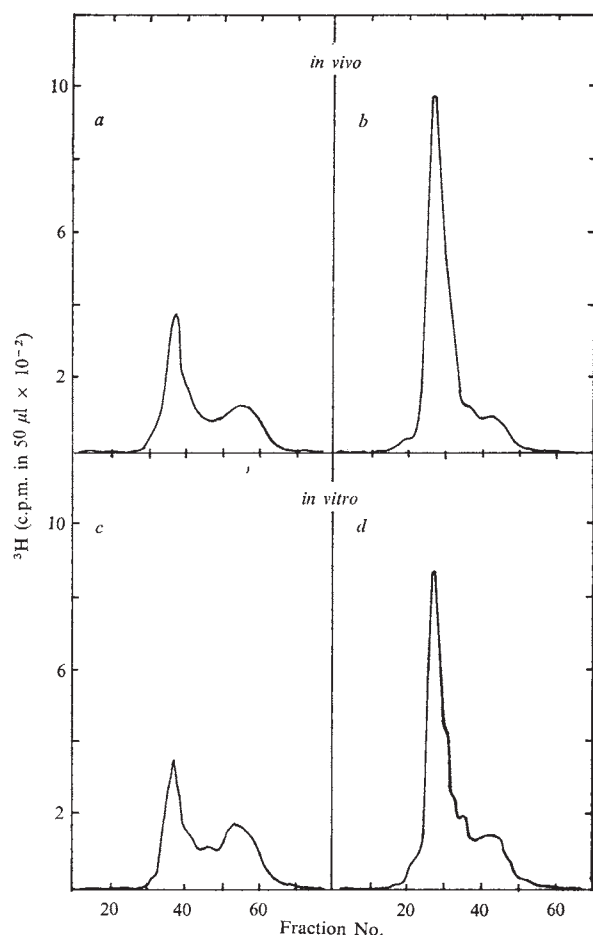


FIG. 5 Pycnographic analysis of DNA labelled *in vivo* (a and b) with ^3H -TdR, or *in vitro* (c and d) with ^3H -dTTP. At 3 h (a) and 7 h (b) after infection, cells were exposed to ^3H -TdR ($5 \mu\text{Ci ml}^{-1}$ in Eagle's medium) for 45 min at 37°C . The medium was decanted, the cell sheet washed with 3 ml phosphate buffered saline and cytoplasm removed by treatment with 0.5% NP40. The nuclei were scraped off and DNA extracted as described in Fig. 2. Nuclear monolayers were also prepared 3 h (c) and 7 h (d) after infection, labelled with ^3H -dTTP ($20 \mu\text{Ci ml}^{-1}$) for 45 min and DNA extracted as before. ^{32}P -labelled phage T4 DNA was added to the DNA thus obtained and the solutions adjusted to a density of 1.723 g ml^{-3} with CsCl and centrifuged in a Spinco Ti 50 rotor for 3 d at 40,000 r.p.m. Five-drop fractions were collected and the acid-insoluble radioactivity to $50 \mu\text{l}$ samples determined. The bottom of the tube is on the left and viral DNA bands at the denser position.

on some occasions than on others. It is accentuated by omissions from, or additions to, the incorporation mixture (Fig. 3). The various components (probably five in number) seem to occur at fairly consistent densities but in varying amounts and, so far, remain unexplained. Some have buoyant densities greater than virion DNA and others have densities intermediate between virion and cellular DNA. It has not yet been determined which peaks are viral, and which cellular, in origin. They may arise from both sources and exhibit greater buoyant densities due to the presence of RNA sequences and/or some degree of single strandedness. Attempts to distinguish between these possibilities have been unsuccessful. The main 'viral' DNA peak is most affected by the omissions; the host peak much less so. At this stage, however, it is still not clear whether these components are intermediates in viral DNA synthesis or merely artefacts.

DNA synthesis was studied in nuclear monolayers prepared from cells infected with *ts* mutants of HSV type II. Mutants of HSV type I were not used because it was difficult to obtain good titres in these cells. (This problem has now been

largely overcome.) The type II mutants (isolated by Timbury¹⁷ and characterised by Halliburton and Timbury¹⁸ in this Institute) were therefore used, although these have certain drawbacks. The switch off of host cell DNA synthesis is poor, the viral eclipse phase tends to be protracted at the permissive temperature (31°C) and the wt virus itself is slightly inhibited at the non-permissive temperature (38°C).

Cells were infected with either of two mutants of HSV II; *ts5*, a DNA-positive mutant (in which viral DNA synthesis appears to be unimpaired at 38°C) and *ts1*, a DNA-negative mutant (in which no viral DNA is made at 38°C). After 7 h at 38°C , when DNA synthesis in cells infected with *ts5* was known to be proceeding rapidly, both batches of cells were cooled to 31°C and *in vivo* DNA synthesis was measured by a 45 min pulse of ^3H -TdR. At the same time nuclear monolayers were prepared and exposed to ^3H -dTTP for 45 min also at 31°C . The labelled DNA was isolated and banded on a CsCl gradient (Fig. 6). The absolute amounts of radioactivity *in vivo* and *in vitro* cannot of course be compared directly, but the *in vitro* profile mirrors the *in vivo* picture in both cases. Thus, in *ts5* infected cells, where viral DNA synthesis appears to be proceeding normally, it can be detected *in vitro*. In the case of *ts1*, on the other hand, no viral DNA synthesis can be seen, *in vivo* or *in vitro*, in the 45 min following the change to the permissive temperature. This probably means that under these conditions a step essential for the assembly of the synthetic apparatus and initiation of DNA synthesis is blocked and that there has been insufficient time at the permissive temperature for the production of detectable amounts of viral DNA. In contrast to this is the finding (J. Hay, personal communication) that both *ts5* and *ts1* when grown at the non-permissive temperature produce normal (or greater than normal) amounts of the soluble herpes-specific DNA polymerase assayable in extracts of infected cells.

It is, therefore, of particular interest to know whether, in the case of *ts1*, the DNA synthetic apparatus, once it has been established at the permissive temperature, still exhibits temperature sensitivity and whether this is demonstrable *in vitro*.

Cells were infected with *ts1* and maintained at 31°C for 16 h (when the rate of viral DNA synthesis is highest in this

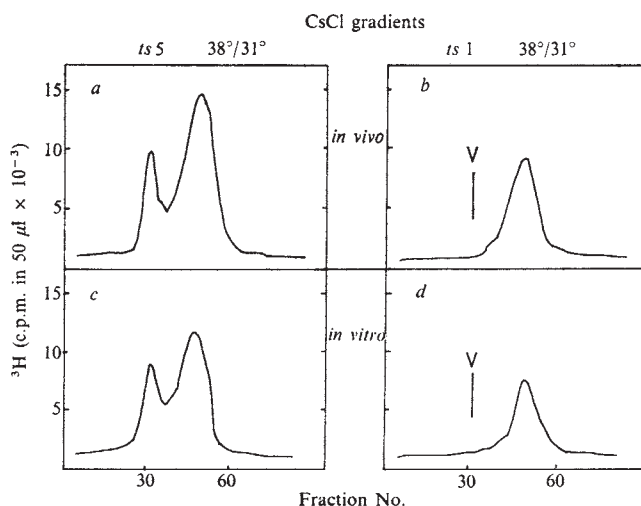


FIG. 6 Pycnographic analysis of the DNA produced *in vivo* (a, b) and *in vitro* (c, d) by *ts* mutants of HSV type II. Cells were infected as described in Fig. 2 with either the DNA-positive mutant *ts5*, or the DNA-negative mutant *ts1*. After absorption, the temperature was raised to 38°C until 7 h after infection when the cells were cooled to 31°C and either labelled for 45 min *in vivo* with (a, b) ^3H -TdR ($5 \mu\text{Ci ml}^{-1}$ in Eagle's medium) or converted to nuclear monolayers and labelled for the same time with (c, d) ^3H -dTTP, as described in Fig. 2. The DNA was extracted and banded as described in Fig. 5.

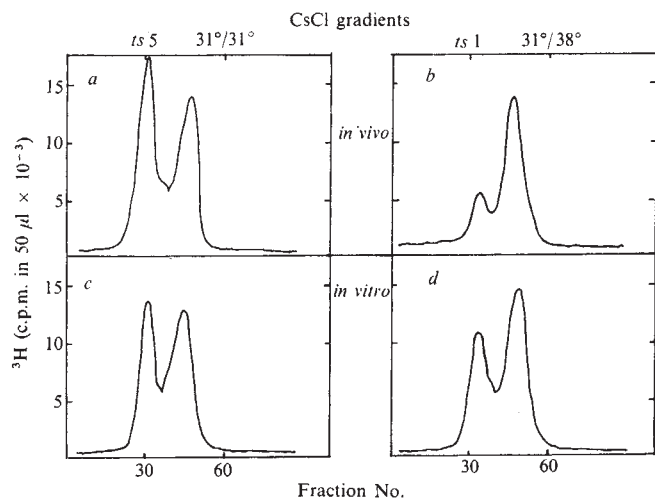


FIG. 7 Pycnographic analysis of the DNA produced *in vivo* (a, b) and *in vitro* (c, d) by HSV II *ts1* at the permissive and non-permissive temperatures. Cells were infected as described in Fig. 2 with the DNA negative mutant *ts1*. At the end of absorption the temperature was lowered to 31° C until 16 h after infection when whole cells, and nuclear monolayers were labelled for 45 min at 31° C, or raised to 38° C for 10 min and then labelled for 45 min at that temperature. The DNA was extracted and banded as in Fig. 5.

mutant). Whole cells and nuclei were then assayed, at both 31° C and 38° C, for incorporation of ^3H -TdR or ^3H -dTTP respectively. (Fig. 7). The viral DNA synthetic apparatus appears to be quite clearly temperature sensitive *in vivo*. The effect *in vitro*, although always in the same direction, is much less marked. This may well mean that the *ts* function contributes less to the overall rate of DNA synthesis *in vitro* than *in vivo*. These experiments are always difficult to control accurately, because of factors such as variation in the synthetic rates at different temperatures, the effect of temperature on the timing of the viral cycle and differences in pool sizes, such that the changes *in vitro* are to some extent masked by the inherent variability of the system. So, although this technique probably gives rise to nuclei which are capable of temperature-sensitive DNA synthesis *in vitro*, further experiments involving a variety of *ts* mutants will be required to confirm this. In addition several important questions remain unanswered. What is the exact nature of the DNA product? What is the role of cytoplasmic factors? Can reinitiation occur?

Nevertheless, these results establish that to a large extent the DNA synthesis observed *in vitro*, in this system, represents true, although perhaps incomplete, viral DNA replication. This method, which enables the quick and gentle exposure of the site of DNA synthesis in a form accessible to precursors, drugs and enzymes, when used with the many *ts* mutants of HSV types I and II now available, can contribute greatly to the elucidation of the molecular biology of herpes virus replication and the problems of mammalian DNA synthesis.

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Multigenic control of ribosomal properties associated with cycloheximide sensitivity in *Neurospora crassa*

It has been shown by McKeehan and Hardesty¹ that, during protein synthesis by 80S ribosomes, cycloheximide selectively inhibits movement of peptidyl-tRNA from the acceptor to the donor site, and that the coupled hydrolysis of GTP, catalysed by the soluble factor T-II in combination with

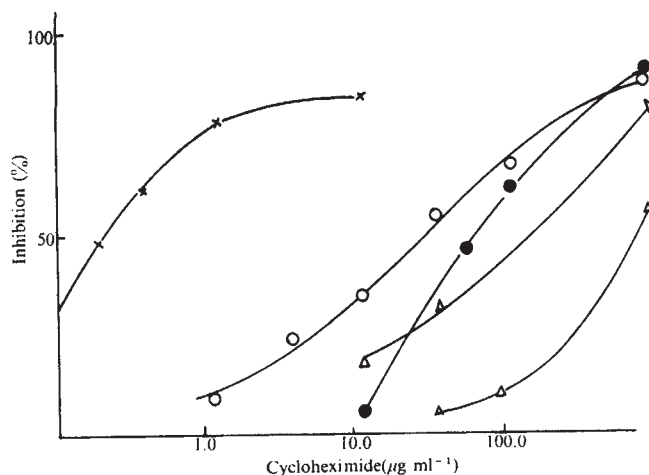


FIG. 1 Effect of cycloheximide on the poly(U)-directed ^{14}C -phenylalanine incorporation into protein by cell free preparation of wild-type and cycloheximide-resistant mutants of *Neurospora crassa*. Protein synthesis *in vitro* was studied in a mixture containing: 50 mM Tris-HCl, pH 7.8; 2 mM ATP; 1 mM GTP; 2 mM phosphoenol pyruvic acid; 15 μg pyruvate kinase; 20 mM KCl; 10 mM mercaptoethanol; 10 mM magnesium acetate; 50 nCi ^{14}C -phenylalanine, specific activity 400 mCi mmole⁻¹; 20 μg poly(U); ribosomes equivalent to 20 μg rRNA and 75 μg of S-100 protein, in a volume of 0.25 ml. Ribosomes washed once in the extraction buffer were used. X, Wild type, STA; O, CH94; Δ , CH40, two different experiments; $\bullet$, CH96.

TABLE 1 Effect of cycloheximide on the wild-type STA strain of *Neurospora crassa* and on three mutants isolated as resistant to the inhibitor

Strains	ID ₅₀ (μ g cycloheximide per ml of medium)*		Protein synthesis <i>in vitro</i>	Resistance on agar (μ g cycloheximide per ml of medium)†	Locus mutated
	growth (dry weight)	Protein synthesis <i>in vivo</i>			
CH40	20.0	15.0	100.0–1000.0	400.0	<i>act-2</i>
CH94	9.0	10.0	25.0	200.0	<i>act-1</i>
CH96	12.0	10.0	50.0	200.0	<i>act-5</i>
STA	0.2	0.3	0.3	1.0	none (wild type)

* The 50% inhibitory dose.

† Threshold concentrations of cycloheximide for inhibition of colony formation on solid medium.

the ribosomes, is not affected. They proposed that cycloheximide might inhibit protein synthesis by occupying sites on the ribosomes usually available for peptidyl-tRNA. As direct association of the T-II catalysed reaction with translocation has not so far been demonstrated, and GTP hydrolysis by T-II is not inhibited by cycloheximide, it is probable that sensitivity to cycloheximide is entirely associated with ribosomes. This was first proposed by Siegel and Sisler² on the basis of species specific differences in the sensitivity to cycloheximide in yeasts and later supported by the work of Wilkie and his associates³. In the latter case a number of cycloheximide resistant mutants have been studied *in vitro*; one of these mutants, having a low level of resistance, seemed to have altered ribosomes.

Resistance to cycloheximide at a high level can result from mutation in any one of at least four genes in *Neurospora crassa*. These genes have different alleles determining different levels of resistance. They are also subject to modification by independent genes⁴. I have investigated three resistant strains, CH94, CH40 and CH96, carrying single gene mutations at the *act-1*, *act-2* and *act-5* loci, respectively, to determine their levels of resistance and the subcellular function which has been modified in each case.

Rapidly collected middle-log phase mycelium, grown from conidia in a New Brunswick Fermentor at 30° C in Vogel's medium⁵, supplemented with 0.1% yeast extract and 2% sucrose, was used for the preparation of the cell free extracts. Preparations of ribosomes and S-100 fractions, as well as assays of the transfer reaction in polyphenylalanine synthesis *in vitro*, were performed essentially as described by Nirenberg⁶. Components of the reaction mixture are indicated in the legend to Fig. 1. Following 10 min incubation at 34° C, 150,000 c.p.m. were detected in hot trichloroacetic acid (TCA)-insoluble material when ribosomes equivalent to 1 mg rRNA were present in the reaction mixture. Dry weight increase in liquid shake cultures during 10 h at 30° C in the presence and absence of cycloheximide, was measured and the % inhibition calculated. Erlenmeyer flasks containing Vogel's medium supplemented with 2% sucrose were inoculated with conidia.

The effect of cycloheximide on protein synthesis *in vivo* was studied in 1 ml samples of 10 h old shake cultures, grown as described above, each incubated at 34° C for 10 min with 20 μ Ci ¹⁴C-phenylalanine, specific activity 10 μ Ci μ mol⁻¹, and cycloheximide. Radioactivity incorporated into protein was measured by the rapid assay system developed by Nirenberg⁶. Sensitivity of growth on solid medium was scored by eye after 2 d incubation at 30° C. Plates, containing Vogel's medium supplemented with 0.3% sucrose, 1% sorbose, and solidified with 2% agar, were spot-inoculated with mycelium.

It is shown in Figs 1 and 2 that in *N. crassa*, mutations in any one of at least three different genes considerably decrease sensitivity to cycloheximide in cell-free extracts, indicating that these genes control components of the protein synthesis apparatus. The effect of cycloheximide on

'hybrid' systems with ribosomes from wild type and supernatant from mutant, or *vice versa*, depends only on the sensitivity of the ribosomal fraction. Thus, the three genes studied in this work control ribosomal components. These mutations are easily scored on agar media because at least a 200-fold increase of cycloheximide concentration is required to inhibit colony formation of the resistant strains in comparison with wild type. Scoring of resistance of growth and *in vivo* protein synthesis in liquid cultures is also easy (Table 1).

It is interesting to notice that in the results obtained in this work the three genes cannot be easily differentiated *in vivo* by level of resistance, in spite of the fact that the decreased sensitivity of all three resistant mutants is clear

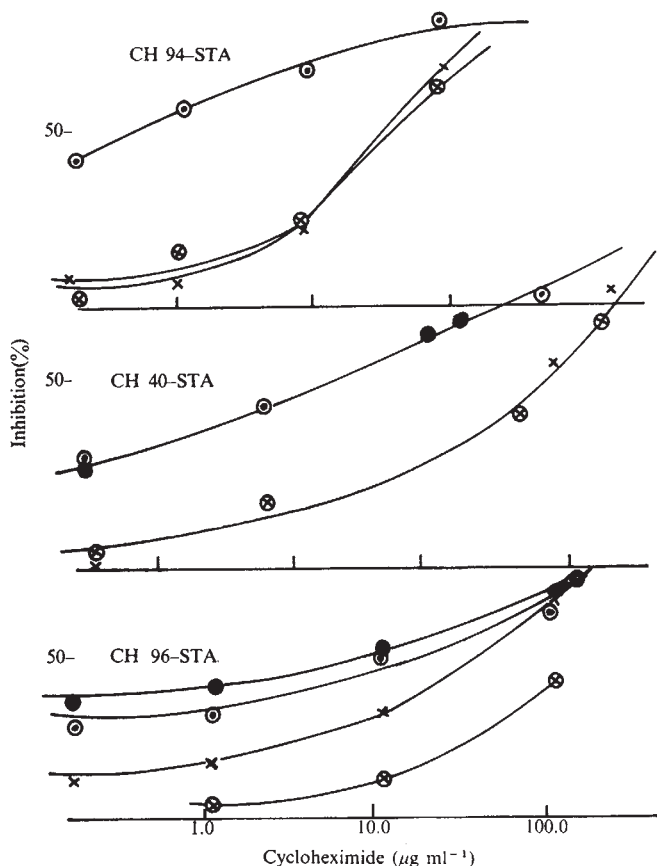


FIG. 2 Response to cycloheximide of cell-free systems of ribosomes and supernatant from wild type and three cycloheximide resistant mutants of *Neurospora crassa*. Protein synthesis *in vitro* was assayed in the mixture described in the legend to Fig. 1, except that ribosomes, washed once in the extraction buffer adjusted to 0.5 M NH₄Cl were used. ●, Wild type system; ×, resistant system; ○, hybrid system with ribosomes of the wild type; ⊕, hybrid system with ribosomes of the resistant type.

cut. *In vitro*, the resistant mutants are even less sensitive than *in vivo* and three different levels of resistance can be recognised, though the *in vitro* resistance of strain CH40 is variable.

The genetic control of resistance of cycloheximide in *N. crassa* was first studied by Hsu⁷. Two unlinked genes, *act-1* and *act-2*, located between well defined markers, have been identified. Hsu has shown that both *act-1* and *act-2* are dominant in heterokaryons and that the double-resistant homokaryotic recombinants obtained from *act-1* × *act-2* are characterised by extremely slow growth rate and almost complete insensitivity to cycloheximide.

My work shows that three genes, two of which are those studied by Hsu⁷, control ribosomal properties related to sensitivity to cycloheximide. It is not yet known whether rRNA or ribosomal proteins are the components modified by the three mutations or whether the genes concerned are structural genes for these components or specify modifying factors as in the case of resistance to kasugamycin in *Escherichia coli*⁸. The dominance of mutations at the *act-1* and *act-2* genes, demonstrated by Hsu⁷, is easily compatible with the idea that these genes specify diffusible factors able to transform all ribosomes in heterokaryons to resistance. On the other hand, if these genes control the primary structure of ribosomal components, their occurrence at scattered sites in the genome is against the idea of more than one of them controlling rRNA. Whatever the modified component may be, however, the extremely slow growth rate of the double resistant (*act-1*, *act-2*) homokaryotic recombinant, observed by Hsu⁷, indicates that some essential function other than binding of the antibiotic is supplied by the normal products of these genes, which is only weakly present in the mutants. That this function is translocation is an interesting possibility.

This work was carried out at the Department of Genetics, University of Leeds, while I held a Scholarship from the Greek State Scholarship Foundation. I wish to thank Professor J. R. S. Fincham for his helpful advice, criticism and support during the course of this investigation and also for critically reading this manuscript.

While this paper was being prepared for publication, Pongratz and Klingmüller reported (*Molec. gen. Genet.*, **124**, 359; 1973) that in *N. crassa*, cycloheximide resistant mutants carrying mutations at the genes *act-1* and *act-2* are resistant *in vitro*.

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Depression of polymorph counts by various scrapie agents

It has been reported by Carp, Licursi, Merz and Merz^{1,2} that the percentage of circulating polymorphonuclear neutrophils (PMN) in C57Bl mice was reduced permanently following injection with either multiple sclerosis or scrapie tissues. They reported that the decrease was first detected 3 d after injection in the case of scrapie and that the decrease persisted throughout the preclinical phase of the disease: most of their results were from mice bled 6 weeks after injection. We report here the confirmation of some of their findings and also discuss certain difficulties which can arise with this test.

Two inbred strains of mice C57Bl, VM and their F₁ cross were used in one mouse colony (ABRO) and C57Bl in another colony (MRI) in various experiments extending in all over 9 months. In one type several biologically different strains of mouse-passaged scrapie³ were used, in a second sheep and goat scrapie (natural or experimental cases) were used, while in a third an attempt was made to confirm the presence of the PMN factor at titres higher than scrapie infectivity and evidence for its replication was sought. In another series of experiments 22A mouse-passaged scrapie was injected into C57Bl, VM and C57Bl × VM mice as a time sequence study from 42 to 158 d. In all experiments etherised mice were bled brachially for PMN counting 6 weeks after injection, or later in the time sequence study.

In all cases intracerebral injections of male mice were used: with the exception of the titrations, inocula were 10⁻² saline brain homogenates (500g, 10 min supernatant). Brains of the appropriate genotype from normal animals or ones with other neurological diseases were used as control inocula. Because it can be much more difficult to recognise different types of leukocytes in mice than in many other species, especially the recognition of PMN with 'tight' nuclei, all the slides were coded and differential counting done 'blind' by Mrs P. Licursi who had made the original observations.

With six different agents tested in ABRO C57Bl mice (22C, 79A, 87A, 51C, 125A and 139A) the PMN percentages were each significantly lower than in the controls ($P < 0.01$) but with 79V agent tested in VM mice the reduction was not significant. Further work is necessary before the important conclusion could be reached that 79V is not associated with any PMN factor. In the time sequence study with 22A agent there was a reduction in each of the three genotypes of mice at 42, 70, 106, 136 and 158 d and this was significant ($P < 0.02$) in 10 of the 15 groups but there was no PMN change in the three groups at 89 d. With six sources of scrapie from sheep (138A, 141A, 161M, RLE, SSBP/1/21, 161P) and two from goats ('scratching' and 'drowsy' types) there were also significant PMN reductions in MRI C57Bl mice compared with the controls which included a pool of 20 brains from normal sheep and sheep with the Border disease or swayback. In the above work mice from both colonies were used and the C57Bl PMN values in control mice ranged from 12.5 ± 0.45 to $16.8 \pm 0.75\%$ whereas the values in the experimental groups ranged from 4.9 ± 0.79 to $10.6 \pm 0.53\%$. Later work was restricted to C57Bl mice in the MRI colony when normal or ME7-scrapie C57Bl brain was used at dilutions down to 10⁻⁸. These attempts to check whether the PMN factor had a higher titre than scrapie infectivity and whether it replicates, failed because the control mice on this occasion had much lower PMN values (5.7–8.8%) than on the previous occasions and were similar to the scrapie-injected groups (5.2–7.7%). Subsequent checks of PMN counts in the MRI colony in uninjected C57Bl mice also showed similar low values (6.6 to 8.9%).

There are two major features in these results. The general findings confirm and extend to numerous strains of scrapie the report of a factor in scrapie-brain which reduces the

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circulating PMN values in mice. There seem to be other unidentified factors, however, which can depress the number of circulating polymorphs for considerable periods of time within a colony. It is probably important that in our earlier group of successful experiments the control C57Bl PMN values were very similar to those reported originally by Licursi *et al.*² It may therefore be necessary for this test to use, as controls, C57Bl mice which have average PMN counts in excess of 12%.

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Can energy generated by sugar efflux be used for ATP synthesis in *Chlorella*?

CELLS of *Chlorella vulgaris* can be induced to accumulate hexose analogues more than one thousand fold^{1,2}. This active uptake can be driven by respiration or photosynthesis^{1,3}. During the steady state of 6-deoxyglucose accumulation, (when sugar influx equals sugar efflux) in the dark with air, only about half the influx is powered by respiratory energy and half the steady-state flux rates are still observed under nitrogen^{3,4}. This part of the steady state flux, can, however, be completely inhibited by uncoupling agents such as dinitrophenol or FCCP (ref. 3). It has been postulated, therefore, that about half of the 6-deoxyglucose steady-state influx is supplied with energy generated by efflux.

But can this form of energy, 'efflux energy', only be used for transport into the cell or can other energy requirements of the cells be met by it? For example, the carrier-mediated efflux of ions (Ca^{2+} or K^{+}) can generate ATP—apparently by the reversal of the energy-dependent influx reaction⁵⁻¹⁷.

To answer this question for *Chlorella* the following experiment was designed. The uptake of glucose and its assimilation is limited by energy under anaerobic conditions in the dark; much higher rates are observed under aerobiosis. When the fate of radioactive glucose in such cells is followed and compared with that of cells preloaded with non-radioactive 6-deoxyglucose, it should be possible to decide if the efflux energy, generated by 6-deoxyglucose leaving the cells, can be used for the ATP-requiring processes of glucose assimilation. *Chlorella* cells were preloaded with unlabelled 6-deoxyglucose until an inside concentration of 0.1 M was reached. These cells were centrifuged and washed with buffer to remove 6-deoxyglucose. Radioactive glucose was added to these cells in anaerobic conditions and the uptake and metabolism of glucose was followed. These data were compared with data obtained with anaerobic cells, not preloaded with 6-deoxyglucose (Fig. 1). Cells not preloaded show a low rate of uptake for glucose of 34 μmol per h per ml packed cells (PC). The glucose taken up is rapidly metabolised; predominantly to glucose phosphate, sucrose and ethanol-insoluble products, which are almost exclusively starch. Hardly any free glucose can be detected.

Cells which were preloaded with 6-deoxyglucose showed a higher rate of uptake (278 μmol per h per ml PC). The rate

of phosphorylation, however, as well as the rate of sucrose and starch synthesis was very similar to that of control cells. Free glucose was considerably accumulated, therefore, and the amount of glucose present after 4 min was clearly higher than the theoretical concentration equilibrium. It has to be emphasised that in no other uptake condition was it possible to observe that *Chlorella* contains a significant amount of free glucose.

The amount of ATP which is required for glucose assimilation is known to be 1 ATP per glucose phosphate, 3 ATP per sucrose and 2 ATP per each glucose incorporated into starch. The ATP utilised for glucose assimilation has been calculated for both experimental conditions and it was found to be identical for non-preloaded cells (51.5 μmol per h per ml PC) and for cells preloaded with 6-deoxyglucose (53.5 μmol h⁻¹ ml⁻¹ PC).

The energy derived from 6-deoxyglucose efflux is quite capable therefore, of driving glucose influx which is increased by a factor 8.18, but it is apparently not able to sustain glucose metabolism by synthesising ATP.

The possibility that 6-deoxyglucose present in the cells prevents the metabolism of glucose, by competitive inhibition for example, has at least been excluded for the hexokinase reaction by *in vitro* experiments.

The results reported here can be explained by the proton symport theory of Mitchell⁹. According to this theory, the proton-translocator movement should be reversible in principle, obeying only the concentration of protons on each side of the membrane. When cells have accumulated high amounts of sugar and the steady state (influx equals efflux) has been reached, protons should also move outward, cotransported with sugar against the proton gradient. In this way sugar efflux would partly restore energy for sugar influx. In addition, any proton gradient, including that created by efflux, could theoretically be transformed to ATP, when a proton dependent ATPase is part of the membrane across which the proton gradient exists. As no additional ATP synthesis has

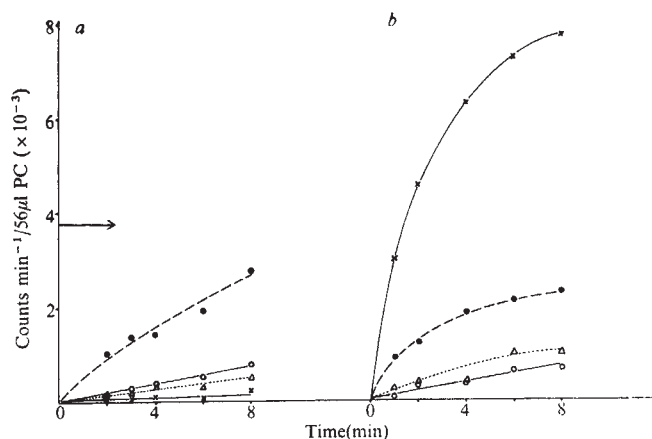


FIG. 1 Uptake and metabolism of ^{14}C -D-glucose by, *a*, *C. vulgaris* and, *b*, by *C. vulgaris* preloaded with 0.1 M 6-deoxyglucose in anaerobic conditions in the dark. Packed cells (PC) 300 μl of induced algae⁴, were incubated anaerobically³ in 12 ml 0.025 M sodium phosphate buffer, pH 6.5. The uptake experiment was started by addition of 0.8 μCi ^{14}C -glucose at a final concentration of 5×10^{-3} M. Samples of 2 ml were withdrawn filtered and the cells were extracted in 80% ethanol. The radioactive compounds were separated by paper chromatography and the amounts determined as described previously⁸. The algae used for experiment *a* did not have detectable amounts of free hexoses inside, whereas the algae for experiment *b* had been incubated aerobically for several hours in 1×10^{-2} M non-radioactive 6-deoxyglucose and were then centrifuged to remove external 6-deoxyglucose. All experiments were performed in the dark at 27°C. ●, Glucose phosphate; ○, sucrose; Δ, insoluble; ×, glucose; →, concentration equilibrium.

been observed, however, this indicates that either no such ATPase exists or that it is not able to compete with the uptake systems for protons. A third explanation might be that the energy available is inadequate.

That a proton gradient might really be the driving force for sugar transport in *Chlorella vulgaris* is indicated by recent results. Experiments with energy poisons suggest that a high-energy intermediate of respiration formed before ATP is the relevant metabolite for active hexose transport in *Chlorella*¹⁰. In addition, it has been possible to observe pH change corresponding to a proton symport, when transportable sugars were added to induced *C. vulgaris* cells¹¹.

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Implications of rigescent integuments as a new structural feature of some algal chloroplasts

CURRENT concepts concerning the origin of plastids have revived the symbiotic theory of Mereschkowski¹ and Faminzin². Evidence in support of this theory has come from recent work on *in vitro* culture of chloroplasts³⁻⁵. We provide further evidence here by the demonstration of a rigescent integument surrounding the chloroplasts of some siphonaceous algae.

During the preparation of chloroplasts from *Caulerpa sedoides*³, we noted that, in distilled water, there was no swelling or bursting of the plastids as might have been expected. It proved very difficult to break them by any of several methods; they withstood blending in a Waring blender, homogenising in a Ten Broek grinder, freezing and thawing, and sonication. The only method to give quantitative disintegration of the chloroplasts was the French pressure cell used at 8,000 pounds inch⁻².

Detergents such as 0.1% Triton X-100 and Tween 80 had no observable effect on the morphology of the chloroplasts and 1% cetyltrimethylammonium bromide (CTAB) removed chlorophyll only after 48 h and several changes of solution. Sodium dodecyl sulphate (SDS) at 1%, removed chlorophyll and internal lamellae rapidly but left an intact rigescent integument inside which was the intact starch sheath around the pyrenoid (Fig. 1). This integument or 'ghost' withstood boiling in distilled water and centrifugation up to 8000g without any observable change occurring in its structure. It also resisted the action of both pronase and lysozyme.



FIG. 1a. Chloroplast of *Caulerpa sedoides* isolated and resuspended in distilled water. $\times 484.50$ Under phase contrast microscopy. b, A rigescent integument prepared from the chloroplasts of *Caulerpa sedoides* by treatment with 1% SDS. The 'ghost' is without chlorophyll and lamellae but retains a characteristic 'butterfly' shape and an apparently unaltered starch sheath around the pyrenoid. $\times 484.50$ Stained with toluidine blue under phase contrast microscopy.

The ghosts were weakly birefringent when examined by polarising microscopy, but scanning electron microscopy showed that they lacked any marked surface features and appeared relatively smooth (Fig. 2). In a related species, *C. cactoides*, transmission electron microscopy and interference contrast microscopy of SDS-treated plastid integuments showed that the terminal body of the plastid, considered by some^{6,7} to be a part of the membranous lamellar system of the chloroplast, appeared to be a coherent and integral part of this integument, and remained attached to it even after treatment with SDS. The role of this terminal structure can be inferred from its behaviour during plastid division, where, early in the formation of the characteristic 'butterfly'-shaped chloroplast, the body seems to divide or duplicate and is thereafter carried in the tip of each 'wing'. This suggests that it is in some way involved in the maintenance of polarity in the chloroplast, which at this stage shows remarkable symmetry and alignment of its lamellar system (Fig. 3). A similar, though smaller and more diffuse terminal body, has been found in the plastids of *C. sedoides*, but its behaviour during division has not yet been followed satisfactorily.

The chloroplast integument has been demonstrated in several species of *Caulerpa* (*C. sedoides*, *cactoides*, and *simpli-cuiscula*) and raises the problem of its possible function. Many saccoglossan opisthobranchs eat species of siphonaceous algae almost exclusively and the primitive forms seem to prefer species of *Caulerpa*⁷. It seems possible that the rigescent integument around the chloroplasts of some of these

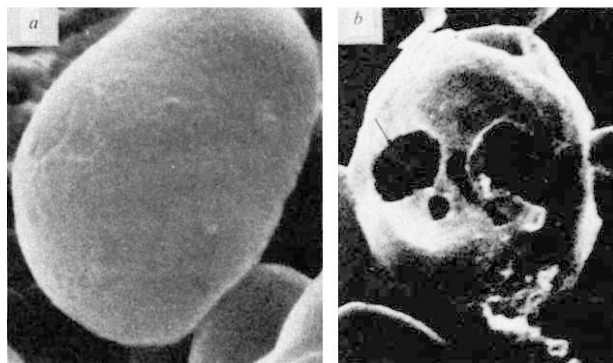


FIG. 2 Exterior surface of rigescent integument of *Caulerpa sedoides* chloroplast as seen with the scanning electron microscope. The surface appears smooth. $\times 5,700$. b, A rigescent integument of *Caulerpa sedoides* chloroplast which was broken during preparation for scanning electron microscopy to reveal not only the starch sheath around the pyrenoid inside the integument (arrowed) but also the 'ghost-like' nature of the integument. The small bodies adjacent to the integument are amyloplasts. $\times 5,700$.

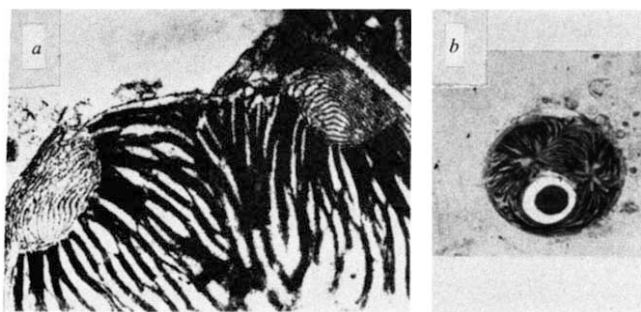


FIG. 3a. An *in vivo* plastid of *Caulerpa cactoides* apparently before division, with the terminal swellings of the integument positioned at the tips of the 'butterfly wings' creating two axes of polarity in the lamellae. $\times 207,480$. b, A chloroplast of *Caulerpa cactoides* showing the remarkable symmetry of lamellae just before division. $\times 2,109$.

species protects them from digestion in the gastric juices of the animal and may be of some selective value in ensuring endosymbiosis in animal tissue.

The discovery of these integuments, however, has a bearing on the endosymbiotic theory of the origin of chloroplasts. Not only do these plastids have an integument which endows them with physical resistance to external conditions, they are also capable of repeated division outside the cell^{4,5}, and have photosynthetic pigments closely related to those of normal green algae and higher plants⁸. The resistance of the integument to the action of lysozyme shows a significant difference from the cell wall material characteristic of the blue-green algae, which have been considered the prime contenders for the role of the primitive chloroplast ancestors. It seems that the plastids of some siphonaceous algae may represent either a missing link in the development of plastids from the ancestral algal symbiont, or an alternative line of plastid evolution.

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Molecular conformation of deoxyguanosine 5'-phosphate

THE allowed conformations of nucleic acids depend on the flexibility of the structure of the nucleotides from which they are constructed. Many X-ray crystallographic studies of the degree of flexibility of these structures have shown that nucleosides exhibit a larger number of preferred conformations than nucleotides, and this led Sundaralingam¹

to suggest that a nucleotide is more 'rigid' than a nucleoside. Berthod and Pullman², however, have drawn attention to the fact that considerations of conformational energy do not imply such a rigid structure.

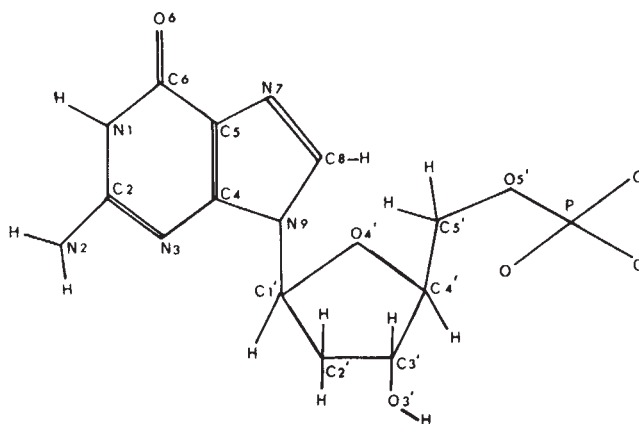
We have recently determined the crystal structure of the disodium salt of deoxyguanosine 5'-phosphate tetrahydrate (GDRP), and we find that the pucker of the sugar ring and also the conformation about the C(4')-C(5') bond are different from those found in other 5'-nucleotides which are normal components of DNA and RNA. These results indicate that a nucleotide may not be as rigid as previous crystallographic studies have implied.

Crystals of GDRP were obtained by evaporation from aqueous solutions. The unit cell is monoclinic with $a = 5.56$, $b = 10.76$, $c = 15.77$ Å, $\beta = 97.9^\circ$, and the space group is P2₁. The structure was solved by Patterson interpretation methods. The positions of the phosphorus atom and one of the sodium atoms were determined from the Harker section, and the orientation of the phosphate group was found by means of a rotation function. Successive Fourier syntheses revealed the positions of the remaining atoms. Details of the structure determination will be given elsewhere. The R factor at the present stage of refinement is 0.06.

The conformation of GDRP about the glycosidic bond C(1')-N(9) is *anti*, with the torsion angle O(4')-C(1')-N(9)-C(4) of 237° . In having the *anti* conformation, GDRP is similar to all other nucleotides in the crystalline state and similar to the majority of nucleosides.

The pucker of the sugar ring is unusual in that relative to the mean plane through the five atoms of the ring, atom O(4') has maximum deviation of 0.25 Å *endo* and atom C(4') has the next largest deviation of 0.21 Å *exo*. This type of pucker has not previously been observed in nucleotides, although it has been observed in the nucleoside dihydrothymidine³.

The conformation about the C(4')-C(5') bond in GDRP is also unusual in that the orientation of the C(5')-O(5') bond relative to the C(4')-O(4') and C(4')-C(3') bonds is *gauche-trans*, with the torsion angle O(5')-C(5')-C(4')-O(4') = 62.6° and O(5')-C(5')-C(4')-C(3') = 175.4° . In all other 5'-nucleotides which are normal components of DNA and RNA, and also in all the refined double-helical models of DNA and RNA, the conformation is *gauche-gauche*. The only nucleotide reported with a *gauche-trans* conformation is 6-azauridine 5'-phosphate (ref. 4), which is not a normal nucleic acid component. Amongst nucleosides, however, the *gauche-trans*, as well as *trans-gauche*, conformations occur quite frequently, but the fact that they had not been observed in nucleotides and dinucleotides was one of the reasons why Sundaralingam suggested that a nucleotide is more rigid than a nucleoside.



The chemical structure of deoxyguanosine 5'-phosphate.

The conformational parameters of GDRP suggest that, depending on the environment, conformations other than *gauche-gauche* are possible about the C(4')-C(5') bond. Indeed, in the study of the binding of nucleotides to lactate dehydrogenase⁵ and to staphylococcal nuclease⁶, the *gauche-trans* conformation is required in order to explain the difference-Fourier maps, and a similar conformation is proposed for a dinucleotide phosphonate when it binds to RNase-S (ref. 7). The present results may also make possible models of single-stranded viral nucleic acids with conformations other than *gauche-gauche*. Finally, it is worth noting that the original Watson-Crick model of DNA⁸ had a *gauche-trans* conformation about the C(4')-C(5') bond, although it was a feature which required modification to *gauche-gauche* in order to fit the X-ray diffraction data⁹.

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Rosette formation by a mouse fibroblast cell line

THE properties and activities of mammalian cell membranes have become an area of increasing interest in cell biology, and their study has involved a variety of experimental approaches. The mutual interaction of membranes from identical or different cell types has often been studied. For example, it has been found that a large proportion of human peripheral blood lymphocytes, when mixed with sheep red blood cells, form cell clusters known as rosettes, in which a central lymphocyte is partially or fully surrounded by red cells¹⁻³. This also occurs with human thymocytes⁴, and is apparently a property of human lymphocytes derived from the thymus (T cells)^{5,6}.

During the course of experiments designed to study the presence of receptors for antigen-antibody complexes on tumour cells⁷, it was found that the mouse fibroblast cell line, A9, made rosettes to sheep erythrocytes which were not coated with antibody. Here we present experiments describing this phenomenon in A9 cells and in cell hybrids between A9 and TLX/5 tumour cells.

Various cultured cell lines were used in this study: (1) A9, a line which lacks guanylic acid-inosinic acid pyrophosphorylase and was originally isolated by Littlefield as a clone of mouse L fibroblasts resistant to 3 $\mu\text{g ml}^{-1}$ of 8-azaguanine⁸. (2) TLX/5, which was derived from a mouse lymphoma⁹ and grown *in vitro*. (3) Three hybrid clones,

resulting from the Sendai virus-induced fusion of A9 and TLX/5 cells *in vitro*. The cell fusion procedure used was essentially that of Davidson¹⁰. The hybrid nature of these cells was confirmed by karyotype analysis. (4) Normal mouse fibroblasts, obtained from secondary cultures of newborn mouse brain. (5) Human skin fibroblasts; (6) A3 hamster fibroblasts obtained from cell lines grown *in vitro* for many generations. The cells used were grown on Falcon plastic Petri dishes in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum and 100 IU ml^{-1} of penicillin, and incubated at 37° C in a humidified atmosphere of 10% CO_2 in air¹¹. Cells were collected from the plates with a rubber policeman and washed twice in gelatin veronal buffered saline (GVB)¹², before being made up to 20×10^6 cells ml^{-1} in GVB (other media such as TC199 have been used with similar results).

Red blood cells were obtained from guinea pig, hamster, horse, human, mouse, rabbit, rat and sheep, usually by collecting whole blood in citrated GVB and washing at least three times. 100 μl of packed red blood cells were added to 10 ml of GVB, which gave a count of approximately 200×10^6 erythrocytes ml^{-1} (this was found to be optimum for rosette formation).

0.05 ml (1×10^6 cells) of the cultured cell suspensions were added to 1 ml of red blood cells in a round-bottomed 5 ml plastic test tube. The cells were mixed and spun down immediately at 150g for 7 min, after which they were resuspended by gently rocking to and fro. 0.33 ml of 2.5% glutaraldehyde in PBS was added to each tube and carefully mixed up to fix the cells¹³. One drop of this suspension was dropped on to a clean slide and allowed to dry. After fixation in methanol, the slides were stained in Leishman's stain and the dried preparations were read¹⁴. In reading the slides, three classes of cells were distinguished; full rosettes were cells which were completely surrounded by erythrocytes, partial rosettes, cells having three or more erythrocytes but were not full rosettes, and non-rosette-forming cells which had no erythrocytes, or one or two, adhering to their surface. Results are expressed as the percentage of these three types.

Table 1 shows the rosette formation of A9 cells with various species of erythrocytes. It can be seen that A9 cells form many rosettes with horse, sheep and hamster, fewer with rat and hardly any at all with the other species tested.

As horse erythrocytes seemed to give more full rosettes than either of the other species tested, we decided to see if any of the other cell lines made rosettes with them. Table 2 shows the results. It can be seen that the only cells which formed any appreciable number of rosettes with horse erythrocytes were A9 and Hybrid C. Two of the hybrids were negative as was the other parental type (TLX/5) and the other cell types tested. This experiment has been repeated using sheep erythrocytes instead of horse erythrocytes with essentially the same results.

The A9 rosettes are sensitive to previous incubation with 0.1% trypsin, or heat killing (56° C, 15 min), but relatively

TABLE 1 Rosetting capacity of A9 cells with various species of erythrocyte

Species of erythrocyte	% Full rosettes	% Partial rosettes	% Non-rosettes	% Full + partial
Horse	46	23	31	69
Sheep	24	53	23	77
Hamster	17	61	22	78
Rat	8	33	59	41
Rabbit	0	10	90	10
Mouse	0	8	92	8
Guinea pig	0	8	92	8
Human	0	4	96	4

TABLE 2 Rosetting capacity of various cell lines with horse erythrocytes

Cell line	% Full rosettes	% Partial rosettes	% Non-rosettes	% Full + partial
A9	39	27	34	66
TLX/5	0	0	100	0
Hybrid A	0	3	97	3
Hybrid B	1	5	94	6
Hybrid C	19	29	52	48
Human skin fibroblasts	0	0	100	0
Mouse brain fibroblasts	0	6	94	6
A3 hamster fibroblasts	0	0	100	0

insensitive to previous incubation with high concentrations of sodium azide.

Many questions are posed by this work. First, since there is a large overlap of cells binding sheep, horse and hamster erythrocytes, is the receptor on the A9 cells responsible for this binding a single receptor, or are there different receptors for sheep, horse and hamster erythrocytes on the same cell?

Second, although A9 cells form rosettes whereas TLX/5 cells do not, only one out of the three hybrid cell lines formed rosettes. One explanation could be that some A9 cells never express the receptor and these were the cells that fused with TLX/5 to form the two negative hybrids. This seems unlikely since the A9 cell line was isolated as a single clone in 1963, and has been cloned several times since then. Another explanation could be that the spatial arrangement of this receptor on the cell surface is critical for red cell attachment. Yet another explanation is that the gene controlling receptor production in A9 was lost during or after fusion with TLX/5, and a corollary to this is that there might be a gene which suppresses receptor production in TLX/5, and that it was this gene which was lost, thus allowing at least one hybrid to show rosette formation. Further observations on the karyotypes of these hybrids should yield information on the possibility of any chromosome loss. The explanations posed above assume that these rosette-forming cells have specific or fairly specific receptors on their surface. The possibility does exist however that rosette formation may be explained by a non-specific phenomenon relating to the physiological conditions of the cells at the time of collection.

Although the A9 cells and one hybrid are the only cells shown here capable of forming rosettes, it might be possible to find other surface markers that are characteristic of certain cell types, such as the lymphocyte in man which binds sheep erythrocytes¹⁻³; this has been suggested as a marker for thymus-derived cells⁵⁻⁶, and is now used routinely in the assessment of T cell function in man¹⁵.

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Antibody coated erythrocytes as a manifold probe for antigens

A variety of antigens can be coupled to erythrocytes and these antigen coated erythrocytes (Ag-E) are widely used for the detection of the corresponding antibody¹. In principle, if erythrocytes were coated with antibody, these antibody-coated erythrocytes (Ab-E) would be useful for the detection of the corresponding antigen. Ag-E and Ab-E should exhibit parallel reversed immunological phenomena, for example, whereas Ag-E are agglutinated and lysed by antibody, Ab-E should be agglutinated and lysed by antigen; whereas Ag-E

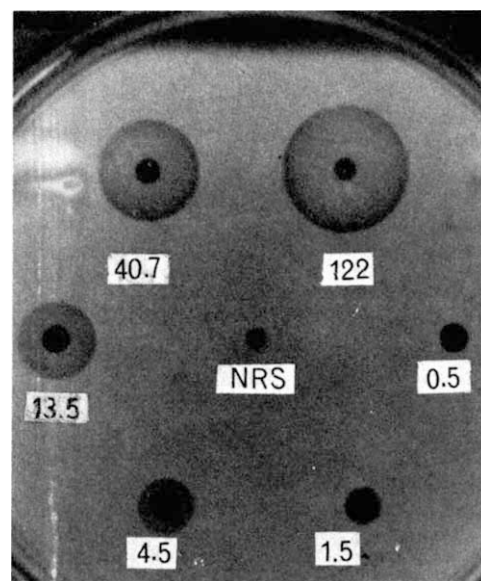


Fig. 1. Disks of hæmolysis produced by human immunoglobulin diffusing into gel containing anti-human immunoglobulin Ab-E. Eleven milliliter of 1% Ab-E and 0.8% agarose in saline borate buffer were allowed to gel in a 10 cm Falcon Petri dish. Five μ l of human IgG diluted in normal rabbit serum (NRS) absorbed with sheep erythrocytes were placed in punched wells. The dish was kept at 4° C for 48 h, then incubated with 4 ml of 1:100 dilution of rabbit anti-human Ig antiserum at 4° C overnight and finally treated with 4 ml of 1:10 dilution of guinea pig complement at 37° C for 2 h. The concentrations (μ g/ml) of human immunoglobulin in the wells are indicated in the photograph.

TABLE 1 Immunoglobulin allotype (antigen)-induced agglutination of sheep erythrocytes coated with antibody specific for rabbit kappa chain allotypes

Indicator cells	Antigen	-1	-2	-3	-4	-5	-6	-7	-8	-9
Anti-b4 Ab-E	b4 serum	-	-	+	+	+	+	+	+	-
	b5 serum	-	-	-	-	-	-	-	-	-
	b4b5 serum	-	-	+	+	+	+	+	±	-
	b4 IgG (10 mg ml ⁻¹) ₄	-	-	+	+	+	+	+	+	-
	b4 serum + anti-b4*	ND	ND	ND	ND	-	-	-	-	-
Anti-b5 Ab-E	b4 serum	-	-	-	-	-	-	-	-	-
	b5 serum	-	-	+	+	+	+	+	+	-

The antigens were absorbed with sheep erythrocytes and serially diluted with 0.2% gelatin in 0.15 M NaCl. Then, 0.1 ml of the dilutions was incubated with 0.1 ml of 1% indicator cells (Ab-E) in 0.15 M NaCl at 4° C overnight.

* 0.1 ml of the dilution of b4 serum was incubated with 5 µl of anti-b4 antiserum at 37° C for 30 min before incubating with the Ab-E.

are antibody reactive, Ab-E should be antigen reactive. To test this prediction, we attached various antibodies, purified from antisera by using immunosorbents², to sheep erythrocytes by the chromium chloride method³. These Ab-E were tested with the corresponding antigens by agglutination, haemolysis and immunocytoadhesion methods.

Erythrocytes were coated with the following purified antibodies: goat anti-rabbit immunoglobulin, goat anti-rabbit α_1 -macroglobulin, rabbit anti-bovine serum albumin (BSA), rabbit anti-mouse immunoglobulin, rabbit anti-human immunoglobulin and rabbit antibodies to various rabbit immunoglobulin allotypes (b4, b5, a1, a2 or a3). These Ab-E were incubated with serial dilutions of the corresponding antigens. In all instances, the antigen agglutinated the erythrocytes coated with the antibody specific for the tested antigen. For example (Table 1), in the system with the rabbit b4 immunoglobulin as antigen and anti-b4 Ab-E as indicator cells, rabbit serum or purified IgG having the b4 antigenic determinants agglutinated anti-b4 Ab-E, whereas b5 serum did not. Similarly, b5 serum, but not b4 serum, agglutinated anti-b5 Ab-E. Moreover, anti-b4 antiserum inhibited the agglutination of anti-b4 Ab-E induced by b4 molecules. This antigen-induced agglutination of Ab-E was characterised by a prozone in antigen excess and by end-points at antigen concentrations as low as 1 ng ml⁻¹ or lower (Table 1). In addition, b4 IgG-E and anti-b4 Ab-E co-agglutinated when incubated together, whereas no agglutination was obtained when b5 IgG-E were incubated with anti-b4 Ab-E (not shown).

Ab-E were first treated with the corresponding antigen, then with antibody specific for the bound antigen and finally with complement. The reaction with the corresponding antigen made the Ab-E sensitive to lysis. Thus, anti-b4 Ab-E lysed when sequentially treated with b4 IgG, anti-b4

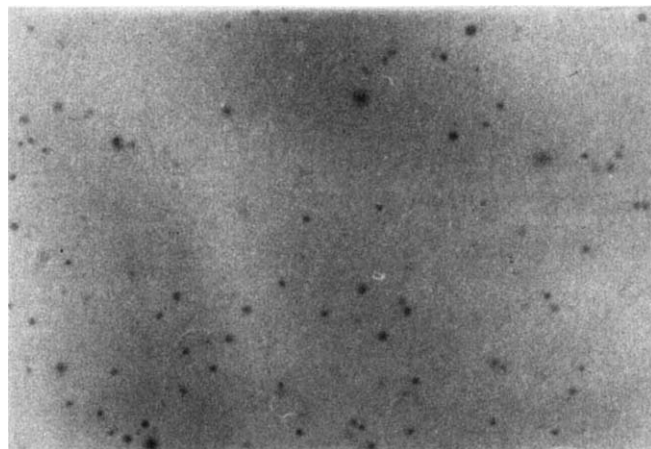


FIG. 2 Haemolytic plaques formed by human lymphocytes with antihuman immunoglobulin Ab-E. Lymphocytes, isolated from blood of a healthy individual by the Ficoll method⁸ were washed with Hanks solution and tested with anti-human immunoglobulin Ab-E by the PFC assay⁹. After incubation at 37° C for 1 h, the dishes were treated with rabbit anti-human immunoglobulin antiserum and guinea pig complement as described in Fig. 1.

antiserum and complement (Table 2). The lysis was specific.

A useful application of this antigen-mediated lysis of Ab-E is the quantitation of antigen by single radial immune haemolysis in gel. When human immunoglobulin at known concentrations was allowed to diffuse from wells into agar gel containing anti-human immunoglobulin Ab-E, localised haemolysis developed around the wells upon addition of rabbit anti-human immunoglobulin antiserum and complement (Fig. 1). At low antigen concentrations, the area of these haemolytic circles, after 48 h, was proportional to the concentration of the antigen. This single radial immunohaemolysis method is a modification of Mancini's method⁴, the major difference being that the antibody is immobilised in the gel by anchorage to the E and the major advantage being that the sensitivity (0.1 µg ml⁻¹) is greater.

The most important practical use of the antigen-mediated lysis of Ab-E is the detection and enumeration of antigen-secreting cells by an unprecedented PFC (plaque forming cell) assay. In all other PFC assays an antigen either native⁵ or artificial⁶ on the membrane of the erythrocyte is the target for the antibody secreting cells. If Ag-E can detect antibody-secreting cells, Ab-E should detect antigen-secreting cells. More specifically, whereas erythrocytes coated with BSA are an indicator for anti-BSA antibody-secreting cells, anti-BSA Ab-E should be an indicator for BSA-secreting cells. We have indeed found that normal human peripheral lymphocytes formed haemolytic plaques when assayed with anti-human immunoglobulin Ab-E (Fig. 2). Being based

TABLE 2 Immunoglobulin allotype (antigen)-mediated lysis of sheep erythrocytes coated with antibody specific for b4 light chain allotype

Indicator Cells	Antigen	Developing Antiserum	Lysis
anti-b4-E	b4 serum	anti-b4	+
	b4 serum	-	-
	b4 IgG	anti-b4	+
	b4 serum	anti-b9	-
	b9 serum	anti-b4	-
uncoated erythrocytes	b4 serum	anti-b4	-

Two ml of 1% Ab-E and 0.8% agarose in 0.15 M NaCl were allowed to gel in a 60 mm plastic Petri dish. All the sera were absorbed with sheep erythrocytes and serially diluted. A droplet of each serial dilution of antigen was placed on the gel and allowed to be absorbed. Then 2 ml of a 1:100 dilution of developing antisera were added. After overnight incubation at 4° C the developing reagent was decanted and 1 ml of a 1:10 dilution of guinea pig complement was added. The dishes were then incubated at 37° C for 1 h. The lowest concentration of b4 IgG, still mediating lysis of the Ab-E, was 1 µg ml⁻¹.

TABLE 3 Rabbit lymphocyte surface immunoglobulin allotypes detected by rosette formation with sheep erythrocytes coated with antibody specific for the b4 and b5 kappa chain allotypes

Rabbit # and genotype	Indicator cells	Inhibitor*	Rosette forming cells (%)
M302 b ⁴ b ⁴	anti-b4-E	—	22
	anti-b5-E	—	0
M341 b ⁵ b ⁵	anti-b4-E	—	2
	anti-b5-E	—	38
	anti-b5-E	b4 serum	33
	anti-b5-E	b5 serum	4
	anti-b5-E	b4, b5 serum	1
	anti-b5-E	anti-b5 antiserum	6
	uncoated-E	—	1

Lymphocytes isolated from heparinised blood by the Ficoll method⁸ were washed thrice with saline, and resuspended to 3×10^6 cell ml⁻¹. Then 0.1 ml of the cell suspension was incubated with 0.1 ml of 1% Ab-E saline containing 0.2% gelatin and 0.05 M NaNa₂ at 4° C overnight. Rosetted and nonrosetted lymphocytes were counted in a haemocytometer.

* Inhibitor (5 μ l) was added to the cell suspension (0.1 ml) before the addition of Ab-E.

on the antigenic properties of the secreted Ig, the assay can be applied to the enumeration of sub-populations of Ig-secreting cells by exploiting the isotypic, allotypic or idiotypic antigenic determinants; for example, allotype secreting cells were enumerated by using erythrocytes coated with the appropriate anti-allotype Ab-E⁷. These immunoglobulin antigen-secreting cells were found in unimmunised individuals, whereas conventional PFC are found only in immunised individuals. Thus, in our PFC assay, an immunoglobulin-secreting cell formed a haemolytic plaque independently from the antibody specificity of the secreted immunoglobulin. This antigen-mediated PFC assay has some distinctive features compared to the conventional antibody mediated PFC assay: (1) the target on the erythrocytes is an antibody rather than an antigen; (2) the lysis of the erythrocytes is mediated by antigen rather than antibody; (3) the cell producing the haemolytic plaque need not be an antibody-secreting cell. Cells other than lymphoid can be tested for secretion, provided that the secreted substance is antigenic. Therefore, this new PFC assay is theoretically a general method for detecting antigen-secreting cells, regardless of the cell type. The method differs from the cytochemical and cytoimmunochemical methods in probing for secretion rather than intracellular presence of antigen.

Peripheral lymphocytes from homozygous b4 or b5 rabbits were incubated with anti-b4 or anti-b5 Ab-E (Table 3). Lymphocytes from a b4 rabbit rosetted with anti-b4 Ab-E (Fig. 3), but not anti-b5 Ab-E. *Vice versa*, b5 lymphocytes rosetted with anti-b5 Ab-E but not anti-b4 Ab-E or uncoated E. Moreover, this rosette formation of b5 lymphocytes with anti-b5 Ab-E was inhibited by b5 (but not by b4) serum by blocking of the anti-b5 combining sites on the erythrocytes. A similar inhibition was induced by anti-b5 antiserum by masking of the b5 specificities on the lymphocytes (Table 3). The rosetting of antigen bearing cells with Ab-E is a direct simple method for the detection of cell surface antigens and for the enumeration of antigen bearing cells.

In conclusion, we have artificially coupled to erythrocytes various purified antibodies. The specificity of the antibody bound by erythrocytes was retained, as shown by the reactivity of these Ab-E with the corresponding antigen either in soluble form (antigen-induced agglutination and antigen-mediated lysis of Ab-E) or bound to the cell surface (rosette formation with Ab-E). Therefore, Ab-E is a probe for antigen. Accordingly, we have shown that Ab-E can be used in new methods for (1) semi-quantitation of antigen by agglutination, (2) quantitation of antigen by single radial immunohaemolysis (manuscript in preparation), (3) enumeration of antigen secreting cells by a PFC assay⁷, (4) enumeration of cells bearing surface antigen by an immunocytadhesion method⁷. The same preparation of Ab-E can be used for all of these assays.

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Inheritance of a recombinant HL-A haplotype and genetics of the HL-1 region in man

THE major histocompatibility system of man, designated HL-1, includes a series of closely linked genes of diverse functions¹. HL-1 includes loci controlling two closely linked genes for the segregant series of HL-A antigens, genes responsible for the stimulation in mixed lymphocyte reactions (MLR-S) and possibly a genetic determinant(s) important for the induction of delayed-type hypersensitivity responses (HDR)^{2,3}. Recombination between the genes for HL-A and between HL-A and MLR-S has been reported^{2,3}.

HL-A types and mixed lymphocyte culture reactions of a Caucasian family (designated Hea) reported here provide further information on the relationship between genes in the HL-1 region. The transmission of a recombinant HL-A type

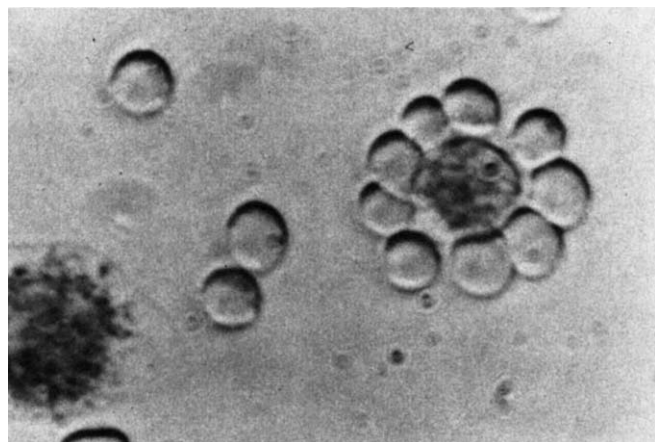


FIG. 3 Microscopic photograph of a rosette formed by a b4 lymphocyte with anti-b4 Ab-E. See Table 3 for experimental details.

TABLE 1 Mixed lymphocyte cultures of the Hea family

Identification	Responder HL-1 genotype or HL-A phenotype	Stimulators										Negative controls	
		I _{1m}	I _{2m}	II _{4m}	II _{1m}	II _{3m}	II _{5m}	II _{7m}	X _{1m}	X _{2m}		R	S†
Father (DH) I ₁	11-7v/3-7x	709*	6,023	3,303	3,307	3,289	2,115	2,123	5,889	7,557		651	228
Mother (EH) I ₂	3-7y/2-12z	10,280	888	8,648	7,225	9,505	5,101	6,337	13,321	9,408		531	311
Sibling (VH) II ₄	3-7x/2-12z	2,396	4,980	803	2,783	7,985	5,684	5,362	10,556	12,806		598	145
Sibling (KH) II ₁	11-7v/2-12z	1,903	4,921	3,542	469	6,654	3,947	3,629	6,602	10,982		556	272
Sibling (VH) II ₃	11-7v/2-7y	2,893	7,870	16,416	10,446	812	870	822	2,012	16,088		465	342
Sibling (CH) II ₅	11-7v/3-7y	2,955	7,661	12,144	3,267	800	588	826	1,490	15,466		569	139
Sibling (DH) II ₇	11-7v/3-7y	4,410	9,218	16,076	7,973	1,677	1,187	1,545	3,052	3,212	1,782	213	
Unrelated (DK) X ₁	2, 10, 8, W18	4,435	10,867	18,227	12,800	1,911	1,855	2,074	960	22,624	1,782	213	
Unrelated (MD) X ₂	1, 2, W17, W18	13,574	8,403	21,189	16,205	18,638	14,262	14,155	20,929	523	389	358	

* Mean of triplicate determinations of ³H-thymidine uptake by a mixture of 1 × 10⁶ responding and 1 × 10⁶ mitomycin-treated stimulating cells.

† In the negative controls R is the mean of triplicate cultures in c.p.m. of radioactive thymidine uptake of 1 × 10⁶ lymphocytes cultured alone for 120 hs. S is the mean of triplicate cultures in c.p.m. of radioactive thymidine uptake of 1 × 10⁶ lymphocytes treated by mitomycin and cultured alone for 120 hs.

Enclosed in the rectangle are the values obtained from the reactions of three siblings from the Hea family and one of the unrelated individuals. The low values obtained in those interactions contrast with the stimulation given by other family members.

from the propositus to her progeny confirms the existence of crossing over between the two *HL-A* loci. The MLR reactions between the recombinant and her siblings furnish additional examples of the importance of differences at the second *HL-A* locus for stimulation in mixed lymphocyte culture. Also, the MLR reactions of family Hea provide three examples of strong stimulation between phenotypically identical pairs of subjects, with each pair having one chromosome identical by descent, and presumptive evidence for genetic control of the level of stimulation with cells from an unrelated subject.

HL-A antigens were detected with a standard two-stage, dye exclusion microcytotoxicity test⁴ using 115 well characterised antisera able to detect all defined *HL-A* and most *W* specificities. Tests were repeated at least three times and *HL-A* haplotypes were assigned by conventional methods². Lymphocytes were collected from the subjects into heparinised (25 U ml⁻¹) Pyrex tubes. Part of the sample was transported to the laboratory as whole blood, and part was diluted with an equal quantity of Eagle's minimal essential medium (MEM) and later divided into two fractions which were processed separately. The MLR were carried out as previously described⁵ in triplicate on each of the three cell samples. The final culture volume of 0.2 ml contained 1 × 10⁵ responding and 1 × 10⁵ (mitomycin-treated) stimulating lymphocytes. After 120 h of incubation, cultures were labelled for 18 h with 0.5 μCi ³H-thymidine (New England Nuclear Corp., NET-027 ³H-thymidine-methyl, (NEN) 1.00 mCi ml⁻¹, specific activity 6.10 mmol⁻¹, 0.036 mg). Cells from all donors survived handling when transported as whole blood. When shipped diluted in MEM, cells from one donor were unresponsive, but the others were reactive. Agreement between results of the various fractions, with this one exception, was excellent. The complete data obtained with cells from whole blood are presented in Table 1. The family studied consisted of sixteen members in three generations. Red cell typing gave no evidence of mixed paternity.

The *HL-A* genotypes of the three generations (Fig. 1) show several interesting features. (1) Subject II 3 gave evidence of recombination within the maternally derived haplotypes 3-7/2-12; the recombinant *HL-A* 2-7 haplotype was transmitted to subject III 3. (2) Lymphocytes from the recombinant (II 3) failed to stimulate unidirectional MLR with cells from siblings II 5 and II 7 who differed from her with respect to first segregant series marker *HL-A* 3 (Table 1). Conversely there was stimulation with cells from subject II 1, who differed with respect to the second series marker *HL-A* 12. (Siblings II 5, II 7 and II 1 all share the paternal 11-7 haplotype with II 3 but differ from her in having intact maternal 3-7 or 2-12 haplotypes from which the recombinant was derived.) (3) Lymphocytes from an unrelated subject (DK) stimulated feebly in two tests and failed to

stimulate in the third test with cells from the three MLR identical sibs II 3, II 5 and II 7, while stimulating adequately with all other family members tested. (4) Stimulation developed between subject I 1 (the father) and his phenotypically identical children II 5 and II 7. Stimulation was also observed between the mother I 2 and her phenotypically identical child II 4. To distinguish the *MLR-S* alleles in the *HL-1* haplotypes of the first generation, designations *v*, *x*, *y* and *z* are given in Fig. 1. Alleles introduced in the second generation are not so distinguished. The transmission of the first generation haplotypes can be readily traced.

To explain the strong stimulation observed between a pair of siblings who were genotypically identical for *HL-A*, Plate *et al.* suggested the existence of a locus outside the *HL-A* region and separable from it by recombination⁶. Yunis *et al.* identified another family in which an individual stimulated with his *HL-A* identical sibling^{2,7}. Analysis of the reactions of several families, each including a known recombinant between two segregant series of *HL-A*, showed that stimulation was consistently associated with differences at the second series and not with differences at the first series^{2,8,9}. The recombinant in the family we have reported stimulates with

HL-1 Haplotypes of the Hea Family

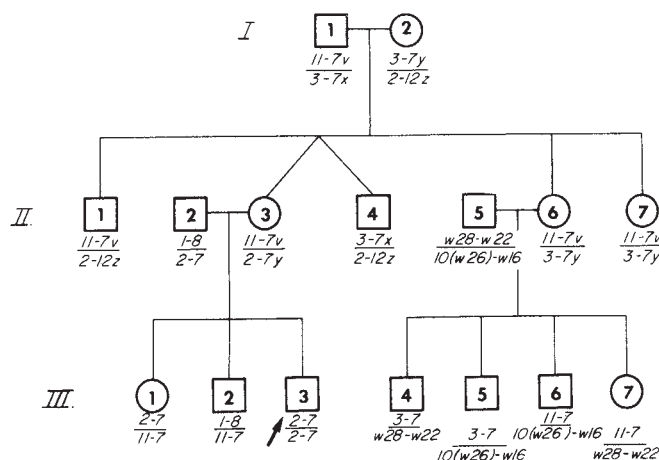


Fig. 1 *HL-1* haplotypes of the Hea family. *HL-1* haplotypes refer to the chromosomal region including two *HL-A* loci, and one *MLR-S* locus (*v*, *x*, *y* and *z*). The hypothetical *HDR* locus is not shown in the pedigree. The arrow identifies the individual to whom the *HL-A* recombinant was transmitted. Numbers under each member of the family represent *HL-A* antigens; the *W* specificities are in conformity with the terminology adopted at the fifth histocompatibility workshop¹¹.

cells from a sibling differing at the second series locus but fails to stimulate with either of the siblings who differ at the first series region. These findings are consistent with the hypothesis that the gene responsible for lymphocyte stimulation, *MLR-S*, is close to the second locus. If this is correct, one would expect the *HL-A* markers to be a poor guide to stimulation in MLR, since the serological tests for *HL-A* do not identify the hypothetical *MLR-S* product. During evolution of the histocompatibility system, recombination between *HL-A* and *MLR-S* should result in a state of equilibrium between the two systems. Therefore, phenotypically identical unrelated individuals would be expected to stimulate frequently, as indeed they usually do². Conversely, one would expect occasional non-stimulation between unrelated subjects regardless of their *HL-A* phenotypes. This family provides three examples of stimulation between phenotypically identical pairs and three examples of non-stimulation or minimal stimulation between phenotypically distinct pairs. One of the individuals (DK) giving minimal stimulation is unrelated, but the pattern of response to her cells correlates with the segregation of the *MLR-S* region of the *HL-1* in the family.

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Initiation of protein synthesis during lymphocyte stimulation

THE stimulation of protein synthesis that accompanies the activation of peripheral blood lymphocytes by phytohaemagglutinin (PHA) has been shown to be due largely to an increase in the rate of initiation of protein synthesis¹. Such an increase could result from either an increase in the availability of initiation factors or of mRNA. This problem can be approached most directly by comparing the ability of cell-free protein synthesising systems from stimulated and

unstimulated lymphocytes to form initiation complexes with ³⁵S-methionyl-tRNA^{Met}. Such studies in reticulocytes have shown that the limitation of initiation occurring during haemin deprivation is accompanied by a marked reduction in the number of methionyl-tRNA-40S ribosomal subunit complexes present, and enabled the elucidation of the mechanisms involved^{2,3}. We show here that the binding of ³⁵S-methionyl-tRNA^{Met} to both 40S subunits and 80S ribosomes is increased after activation of lymphocytes by PHA. These differences can be overcome by the addition of initiation factors from reticulocyte ribosomes, but not by the addition of exogenous globin mRNA.

Lymphocytes were purified from defibrinated pig blood by sedimentation with dextran, filtration through cotton wool to remove phagocytes and centrifugation on ficoll-hypaque step gradients to remove residual erythrocytes. The lymphocytes were cultured at 2×10^6 ml⁻¹ in Eagle's minimal essential medium supplemented with 10% autologous serum. Incubation was for 20–24 h at 37° C, either with or without 3 µg ml⁻¹ purified PHA.

To prepare cell-free extracts, the cells were collected by centrifugation and resuspended at 10⁹ ml⁻¹ in 10 mM KCl; 20 mM Tris-Cl pH 7.5; 1.5 mM Mg acetate for 10 min at 4° C. They were disrupted with 10 strokes of a Potter homogeniser. The ionic concentration was adjusted to 80 mM KCl; 25 mM Tris-HCl pH 7.5; 4 mM Mg acetate; 6 mM mercaptoethanol (TKM medium), and the homogenate was centrifuged at 6000g for 6 min. The supernatant was passed through Sephadex G25 equilibrated with TKM medium.

³⁵S-methionyl-tRNA^{Met} was prepared by incubation of discharged rabbit reticulocyte tRNA with ³⁵S-methionine

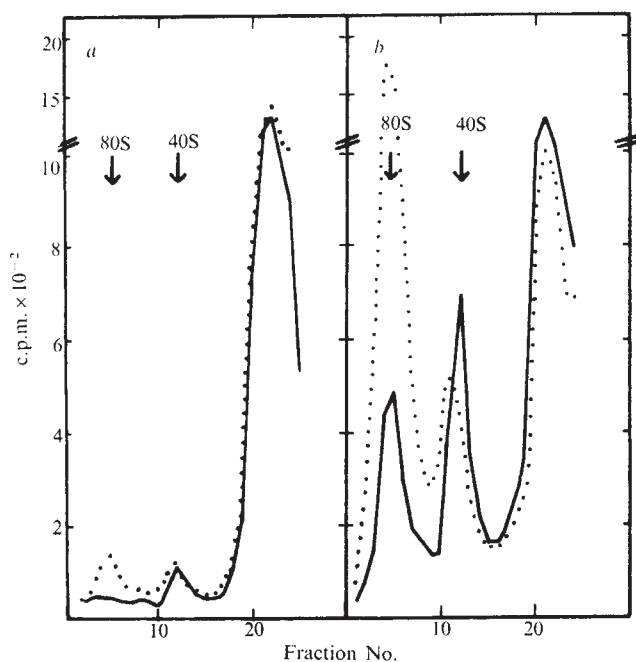


FIG. 1 Effect of globin mRNA on initiation complex formation by extracts from lymphocytes incubated with (b) or without (a) PHA for 24 h. Cell free extracts containing 37 µg RNA were incubated at 30° C for 5 min in 0.2 ml TKM medium supplemented with 1 mM ATP, 0.2 mM GTP, 4 mM creatine phosphate, 0.5 mg ml⁻¹ creatine phosphokinase, a mixture of 19 amino acids excluding methionine, each at 20 µM, and ³⁵S-methionyl-tRNA^{Met} (36,000 c.p.m.). Each incubation was then layered onto a 10–30% sucrose gradient in TKM and centrifuged in the SW 50-1 rotor at 49,000 r.p.m. for 1.75 h. Twenty-six to twenty-eight fractions were collected from each gradient and 1 ml of 2% cetyltrimethylammonium bromide and 1 ml of 0.5 M sodium acetate pH 5.2 containing 0.5 mg ml⁻¹ yeast RNA were added to each. The precipitates were collected on glass fibre filter disks and the radioactivity in each determined. —, Without globin mRNA; ···, with globin mRNA.

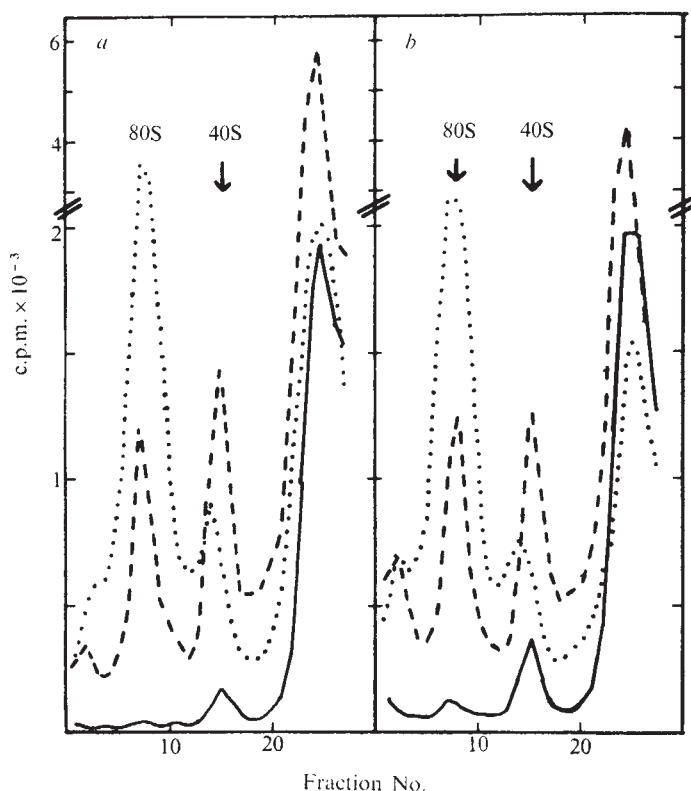


FIG. 2 Effect of reticulocyte initiation factors on initiation complex formation by extracts from lymphocytes incubated with (b) or without (a) PHA for 24 h. The composition of incubation mixtures was as for Fig. 1, except that each contained 19 μ g extract RNA, 72,000 c.p.m. 35 S-methionyl-tRNA, Met and, where indicated, 25 μ g reticulocyte initiation factors. Incubation was at 37° C for 2 min. The sucrose gradients included a cushion of 2 M sucrose, and centrifugation was at 42,000 r.p.m. for 2 h. —, Without initiation factors; ---, with initiation factors; ···, with initiation factors and globin mRNA.

and activating enzymes from *E. coli*⁴. Methionyl-tRNA, Met is not charged under the conditions used. Globin mRNA was prepared as previously described^{5,6}. Initiation factors were extracted from reticulocyte polyribosomes as described by Schreier and Staehelin⁷. The fraction used was that which eluted from DEAE-cellulose between 0.1 M and 0.25 M KCl, and was concentrated by precipitation with ammonium sulphate.

Extracts prepared from PHA-stimulated lymphocytes incorporated considerably more 14 C-leucine into protein than those from unstimulated cells. In both cases at least 90% of the incorporation was due to the completion of polypeptide chains initiated in the intact cell, as judged by its insensitivity to inhibitors of initiation such as aurin tricarboxylic acid and pactamycin. These results were very similar to those obtained previously with ribosomes from stimulated and unstimulated human lymphocytes⁸.

35 S-methionyl-tRNA, Met added to such extracts rapidly became bound to 40S ribosomal subunits, either at 0° C or at 37° C, but there was always more binding to the subunits in the extracts from stimulated cells (Fig. 1). This difference was not due to differential hydrolysis of the 35 S-methionyl-tRNA, as only about 5% was hydrolysed during the incubation period, and the rate of hydrolysis with either extract was not much faster than that with buffer alone.

Little 35 S-methionyl-tRNA became bound to 80S ribosomes in extracts from unstimulated lymphocytes, while there was significant but rather variable radioactivity sedimenting in both the 80S and the polyribosome regions of extracts from PHA-stimulated cells. In some extracts more

methionyl-tRNA was bound to the 80S and polyribosome regions than in the 40S region. The sedimentation of radioactivity in the 80S region was abolished by 10^{-4} M aurin tricarboxylic acid, but not by 3×10^{-3} M cycloheximide.

Addition of globin mRNA increased the binding of 35 S-methionyl-tRNA to the 80S region, but had little effect on the difference between stimulated and unstimulated cell extracts (Fig. 1). The extracts from the PHA-stimulated cells were thus able to utilise the added mRNA more effectively. The amount of radioactivity sedimenting in the 40S region was not much altered by the addition of mRNA, although the peak of radioactivity did sediment a little further down the gradient.

In contrast, the binding of 35 S-methionyl-tRNA to both 40S and 80S regions was greatly increased, and the difference between stimulated and unstimulated extracts almost completely overcome, by the addition of initiation factors from reticulocyte ribosomes (Fig. 2). The binding to the 80S region was abolished by 10^{-4} M aurin tricarboxylic acid or 10^{-5} M edeine. Both these inhibitors prevent the formation of normal mRNA-containing 80S initiation complexes^{9,10}, but do not inhibit the non-specific binding of tRNA to 80S ribosomes, such as occurs at high Mg^{2+} concentrations¹¹. The reticulocyte initiation factor preparation did not contain significant amounts of mRNA, as judged by its inability to cause a 'shift' reaction in sparsomycin-treated reticulocyte lysates¹², so that the mRNA required for the binding of the methionyl-tRNA to the 80S ribosome must be derived from the lymphocyte extracts. Addition of globin mRNA to either type of extract in the presence of reticulocyte initiation factors markedly increases the binding to the 80S region (Fig. 1). In this case the amount of methionyl-tRNA bound to the 40S region, and the amount at the top of the gradient are both reduced, and the slight shift in the sedimentation rate of the radioactivity in the 40S region is more pronounced.

In a previous series of experiments⁸ we showed that after stimulation by PHA, lymphocyte ribosomes contained a higher concentration of those initiation factors required for the translation of the synthetic mRNA poly(U) at low Mg^{2+} concentrations. The results presented here support much more directly the idea that the low rate of initiation in unstimulated lymphocytes is not due simply to a lack of available mRNA, but is due to a lack of one or more of the initiation factors that can be extracted from reticulocyte ribosomes.

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Rosette formation of human erythrocytes on cultured cells of tumour origin and activation of complement by cell membrane

WHEN some cultured cells (OAT cells¹, a cultured cell line from lung cancer) were treated with normal fresh human, guinea pig or rabbit serum and mixed with human erythrocytes (HuE), rosette formation of HuE occurred around the treated cells. We thought initially that the phenomenon might involve natural antibodies and complement because antigen-antibody-complement complexes are known to adhere to HuE by the phenomenon of immune adherence². This possibility was excluded by the finding that rosette formation of HuE around serum-treated cultured cells occurred in the absence of Ca²⁺, since Ca²⁺ is known to be essential for the reaction of the first component of complement (C1). That the alternative pathway of complement activation³ (Properdin system) might underlie the generation of rosette-forming ability was suggested by the demonstration that Mg²⁺ was an essential requirement.

In the following experiments, gelatin-veronal-buffered saline (GVB) containing 0.1% gelatin was used as the diluent. Three modified diluents were prepared to characterise the role of Ca²⁺ and Mg²⁺: (1) Ca²⁺-Mg²⁺-GVB containing 0.15 mM CaCl₂ and 0.5 mM MgCl₂; (2) Mg²⁺-EGTA-GVB containing 10.5 mM MgCl₂ and 10 mM EGTA (ethyleneglycoltetraacetate); (3) EDTA-GVB containing 10 mM EDTA (ethylenediaminetetraacetate).

OAT cells were treated with diluted serum in Ca²⁺-Mg²⁺-GVB, Mg²⁺-EGTA-GVB or EDTA-GVB (Table 1). After treatment with serum at 30° C for 30 min, cells were washed three times with EDTA-GVB and mixed with HuE. After 1 h incubation at 30° C, the cells were examined microscopically, and the percentage of rosette-forming cells were calculated. Cells which bound two or more HuE were regarded as positive.

In the absence of divalent cations (in EDTA-GVB), OAT cells did not form rosettes with HuE. In the presence of Mg²⁺, OAT cells developed the rosette-forming ability even in the absence of Ca²⁺ (Table 1). This differential dependence on Mg²⁺ was also found where guinea pig and rabbit serum were used instead of human serum.

Cultured cells derived from Burkitt's lymphoma (Daudi⁴ and P3HR-1 (ref. 5)), HeLa cells and Chang Liver cells were treated with human serum (at a final concentration of 1:15) in the presence or absence of Ca²⁺ and/or Mg²⁺ as described above.

Daudi cells and P3HR-1 cells were able to form rosettes with HuE following treatment with Mg²⁺-EGTA-GVB but HeLa cells and Chang Liver cells were not (Table 2). In a similar experiment, four cell lines derived from biopsies of nasopharyngeal carcinoma (NPC204, NPC316, NPC415 and NPC428)⁶ all acquired the ability to form rosettes after treatment with human serum in Mg²⁺-EGTA-GVB. Two primary cultures of acute myeloid leukaemia cells were unable to form rosettes.

OAT cells were also treated with C4-deficient guinea pig serum⁷ in the presence or absence of divalent cations. After treatment in the presence of Mg²⁺, OAT cells were found to form rosettes of HuE and also to fix C3 molecules on the cell membrane. C3 was detected by use of ¹²⁵I-labelled (Fab')₂ antibody to guinea pig C3. OAT cells treated with

TABLE 1 Rosette formation of HuE around OAT cells treated with human serum in the presence or absence of Ca²⁺ and/or Mg²⁺

Diluent	Dilution of human serum*			
	1:20	1:60	1:200	0
Ca ²⁺ -Mg ²⁺ -GVB	56†	48	5	0
Mg ²⁺ -EGTA-GVB	43	20	0	1
EDTA-GVB	1	2	0	0

To 0.2 ml 5 × 10⁶ ml⁻¹ OAT in GVB was added 0.1 ml of Ca²⁺-Mg²⁺-GVB, Mg²⁺-EGTA-GVB or EDTA-GVB. To each of these, 0.1 ml of a dilution of human serum in corresponding buffer (Ca²⁺-Mg²⁺-GVB, Mg²⁺-EGTA-GVB or EDTA-GVB) was added. After 30 min incubation at 30° C, the treated OAT cells were washed and resuspended in 0.05 ml of EDTA-GVB and mixed with 0.05 ml of 4 × 10⁷ ml⁻¹ HuE in EDTA-GVB. After 1 h at 30° C, rosette formation of HuE around the treated OAT cells was observed microscopically.

* Final concentration in the above reaction mixture.

† Percentage of OAT cells binding two or more HuE.

C4-deficient guinea pig serum in EDTA-GVB did not form rosettes and did not fix C3. These findings indicated that the cell membrane of cells such as OAT, Daudi, P3HR-1 and NPCs had the ability to activate C3 molecules and to cause them to associate with the cell membrane. It has been reported that trypsinisation of HuE results in loss of the immune adherence receptor⁸. Since trypsinised HuE did not form rosettes on serum-treated OAT cells, a likely interpretation is that the interaction of cell membrane immune adherence receptors and membrane bound C3 is involved in the mechanism of rosette formation. Activation of C3 probably occurs through the alternative pathway³, since the reaction occurred with C4-deficient serum and required Mg²⁺. An antigen-antibody reaction is unlikely to be involved since sheep erythrocytes sensitised with antiserum did not form rosettes with HuE even after the treatment with human serum in Mg²⁺-EGTA-GVB. If it is postulated that the process of rosette formation is based on complement activation by the alternative pathway³, the cell membrane of rosette-forming cells may have some molecular configuration similar to that of bacterial lipopolysaccharides which also have the ability to activate the alternative pathway of complement.

To examine the relationship between the rosette-forming ability and virus infection, the following experiment was performed with acute myeloid leukaemia cells. The cells were found to lack rosette-forming ability following treatment with serum in Mg²⁺-EGTA-GVB. Suspensions of cells were added to spleen extracts of DDD mice suffering from Friend leukaemia, to tissue culture fluid of C3H2K cells infected with Moloney sarcoma virus and to a spleen extract of DDD mice infected with Rauscher virus. Cells were incubated in RPMI 1640 medium containing 20% heat-inactivated a-γ human serum at 37° C under 5% CO₂. After 12, 36, 60, 84 and 108 h incubation, cells were tested for rosette-forming ability.

TABLE 2 Percentage of rosette-forming cells after treatment with 1:15 human serum in presence or absence of Ca²⁺ and/or Mg²⁺

Cells	1:15 dilution* of human serum in		
	Ca ²⁺ -Mg ²⁺ -GVB	Mg ²⁺ -EGTA-GVB	EDTA-GVB
Daudi	62%†	49%	0%
P3HR-1	36%	36%	3%
HeLa	7%	0%	0%
Chang liver	11%	1%	0%

To 0.2 ml of 5 × 10⁶ ml⁻¹ cells in GVB was added 0.2 ml of the appropriate diluent; then they were mixed with 0.2 ml of 1:5 dilution of human serum in the corresponding diluent (Ca²⁺-Mg²⁺-GVB, Mg²⁺-EGTA-GVB or EDTA-GVB). After 30 min incubation at 30° C, the treated cells were washed and reacted with HuE as in the legend of Table 1.

* Final concentration in the reaction mixture.

† Percentage of cells binding two or more HuE.

TABLE 3 Effect of addition of virus-rich materials on rosette-forming ability of acute myeloid leukaemia cells

Materials added	Rosette forming ability* after an incubation period of (h)				
	12	36	60	84	108
Friend virus (Spleen extract)	3	0	4	2	3
Moloney sarcoma virus (Tissue culture fluid)	1	0	2	3	25
Rauscher virus (Spleen extract)	0	1	0	2	3
None	0	0	0	1	1

From heparinised blood of an acute myeloid leukaemia patient, a leukaemic cell fraction was collected and suspended in RPMI 1640 medium with 20% heat-inactivated α - γ human serum† at a concentration of 1.5×10^6 ml⁻¹. To 9 ml of the cell suspension, 0.3 ml of Friend virus fraction (spleen extract), 0.6 ml of Moloney sarcoma virus fraction (tissue culture fluid) or 0.3 ml of Rauscher virus fraction (spleen extract) were added. To the control cell suspension nothing was added. Each mixture was separated into 6 tubes each of which contained 1.5 ml. They were incubated at 37 °C under 5% CO₂. After incubation, cells of each tube were washed three times with GVB and resuspended in 0.4 ml of Mg²⁺-EGTA-GVB. To the cell suspension, 0.2 ml of 1:3 dilution of human serum in Mg²⁺-EGTA-GVB were added and the mixture was incubated at 30 °C for 30 min. After washing with EDTA-GVB three times, cells were resuspended in 0.2 ml of GVB and mixed with 0.2 ml of 2×10^6 ml⁻¹ HuE in EDTA-GVB. Rosette formation of HuE on the cells were observed after 1 h at 30 °C.

* Percentage of cells binding two or more HuE.

† From normal human serum of Type O, γ -globulin fraction was removed by 45% saturation of ammonium sulphate. The α - γ human serum was dialysed against saline to remove ammonium sulphate and then dialysed against foetal calf serum to reconstitute dialysable serum factors before use.

following treatment with human serum in Mg²⁺-EGTA-GVB. Cells which had been mixed with material rich in Moloney sarcoma virus became able to form rosettes after 108 h incubation (Table 3). This finding suggested that rosette-forming ability could be brought about by viral infection. If cells able to activate complement non-specifically are either present or are generated *in vivo*, phenomena simulating immunological reactions may occur without reactions of specific antigens and antibodies. Such a mechanism may be involved in the pathogenesis of changes occurring in some of the so-called autoimmune diseases.

We thank Dr M. M. Frank for his kind gift of C4-deficient guinea pig serum, Dr T. Odaka for fractions of Friend leukemia, Moloney sarcoma and Rauscher leukemia viruses, Dr S. Oboshi for OAT cells and Dr M. Takada for NPC cells.

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Temperature dependence of cation permeability of dog red cells

It has been suggested that changes in the physical state of phospholipids are responsible for the observed dependence upon temperature and membrane composition of the self-diffusion of sodium ions through lipid vesicles¹ and of diverse membrane transport processes²⁻⁴ some of which are mediated by enzymes^{5,6}. This is supported to some extent by the detection of phase-transitions in both natural membranes⁷⁻¹⁰ and in lipid-extracts¹¹⁻¹³ using X-ray diffraction and other techniques. It seems, however, that only membranes of low cholesterol content exhibit any of the usual characteristics of these transitions^{1,9,12,13}.

We report here that the transport of sodium ions into dog red cells, under isotonic conditions, increases as the temperature is lowered from 38° C and reaches a maximum at 22–25° C. By contrast, the influx of potassium ions is described by a normal Arrhenius relationship between 38° C and about 12° C at which temperature a well-defined minimum is reached. This implies that the fluidity of the lipids in the membranes of these cells, which are rich in cholesterol, does influence the fluxes of sodium and potassium. Furthermore, the disparity between the effects of temperature on the fluxes, which are mainly passive in this system, indicates that two independent processes are involved in the ion-transport mechanisms.

Blood was drawn from the jugular vein of unanaesthetised Beagles into syringes wetted with heparin. Within a few minutes it was chilled to 4° C and centrifuged at about 1900g (MSE Minor). Plasma and white cells were aspirated and the red cells were washed three times at 4° C with a solution containing NaCl 142.2 mM, KCl 5.0 mM, Na₂HPO₄ 5.2 mM, NaH₂PO₄ 0.8 mM, CaCl₂ 1.0 mM, MgCl₂ 0.25 mM, glucose 5.0 mM and 1% (w/v) bovine serum albumin (Sigma, fraction V). The washed cells were suspended (5 ml packed cells to 25 ml of bathing medium) in Erlenmeyer flasks which were placed on ice. ¹³¹I-human serum albumin (Radiochemical Centre, Amersham) was passed through an ion exchange resin (Deacidite FF-1P, Permutit) before it was made up in isotonic buffer and added to each suspension (2 μ Ci ml⁻¹).

Cell suspensions were shaken in a water bath at 38° C for 30 min and then transferred to shaking water-baths at the temperature of the influx of ²⁴Na and left for a further 30–45 min at 38° C, or for 1–2 h at lower temperatures. At the end of these incubation periods, 0.2–1.0 ml of buffer solutions containing either ²⁴Na or ⁴²K (Radiochemical Centre, Amersham) were added to each suspension (10 μ Ci ml⁻¹) at 15 s intervals after which the suspensions were agitated vigorously for 2 min. At various times thereafter, 4 ml samples of each suspension were pipetted into centrifuge tubes pre-cooled in ice-water. These were transferred to the cold room (4° C) and centrifuged for 3–5 min. Samples of the supernatants were saved and the remainder aspirated. 100 μ l volumes of the packed cells were taken using a gas-tight Hamilton syringe with a Chaney adaptor (Reno, Nevada) and lysed by addition to 2 ml of water in Pyrex test-tubes. The Hamilton syringe was calibrated and served as the primary reference for the volume of packed cells in each lysate; it was rinsed several times with detergent solution (2% Triton X-100) and water between each pipetting. At temperatures between 20° C and 38° C, samples of red cell suspensions were taken at 30–60 min intervals throughout 3–5 h, but below 20° C samples were taken at less frequent intervals spaced over 12–15 h. The radioactive supernatants were diluted and prepared for γ -counting in the same way as the lysates. The activities of ²⁴Na (or ⁴²K) and ¹³¹I were measured in a two-channel γ -counter (Packard, Model 3002 or LKB Wallac 80000) and the appropriate corrections for

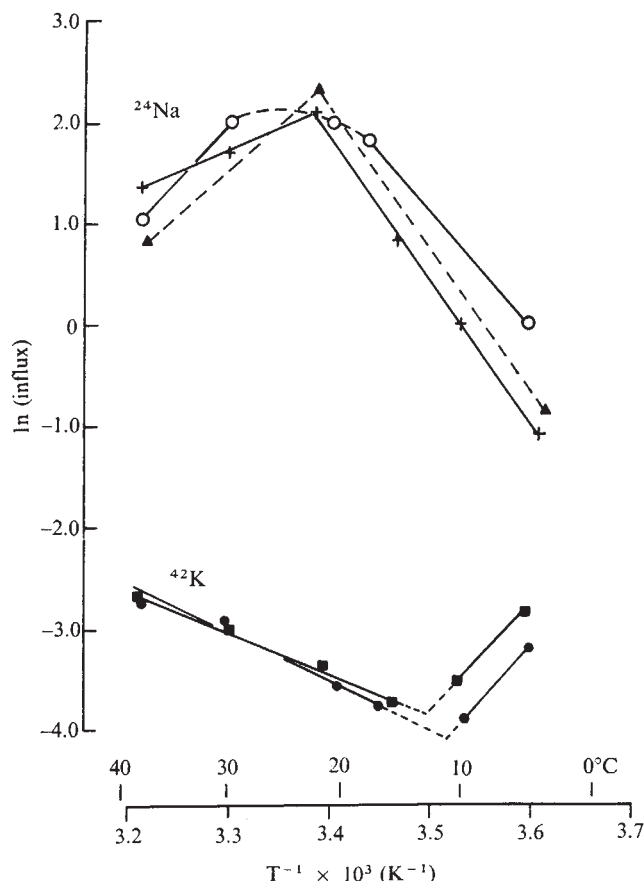


FIG. 1 Arrhenius plots of steady-state fluxes of sodium and potassium in dog red cells. The values of the fluxes (meq per l cells h^{-1}) at 38° C were 2.28 ± 0.35 (+), 3.96 ± 0.10 ($\blacktriangle$) and 2.87 ± 0.25 (O) for sodium, 0.0645 ± 0.0012 ($\bullet$) and 0.0703 ± 0.0049 ($\blacksquare$) for potassium. Blood from a different animal was used in each set of flux measurements. The errors arise mainly from the standard errors of the regression lines fitted by the method of least squares to 3–5 points on an uptake curve. In one experiment (O—O) the cells were not pre-incubated at 38° C as described in the text, but were left in ice for a few hours after washing, and then gently agitated for 1–2.5 h at the temperature of ^{24}Na -uptake before the addition of tracer. The least squares fit to the ascending limb of (+—+) gave a negative apparent activation energy for sodium influx of 8.7 ± 0.3 kcalorie mol^{-1} and for the descending limb a positive value of 28.2 ± 0.6 kcalorie mol^{-1} , one point at 22.4° C appeared to be common to both limbs of the Arrhenius plot. The activation energies for potassium influx between 38° C and 15° C were 8.5 ± 0.3 kcalorie mol^{-1} ($\blacksquare$ — $\blacksquare$) and 9.2 ± 1.2 kcalorie mol^{-1} ($\bullet$ — $\bullet$); below the 'transition' temperature at 11–13° C the value was about -22 kcalorie mol^{-1} .

^{24}Na (or ^{42}K) activity in the ^{131}I channel were made. Finally the concentrations of haemoglobin, sodium and potassium in each lysate were measured. After correcting for tracer in the trapped extracellular volume of the packed cells, it was possible to calculate the ^{24}Na or ^{42}K uptake in terms of activity per unit cell volume, the relative volume of the cells from the intracellular concentration of haemoglobin, and the intracellular concentrations of the cations from which net movements of Na and K could be estimated to check if the cells were in the steady-state. This method for the measurement of tracer uptake in cell suspensions has obvious advantages over the more conventional techniques which involve repeated washing of the cells in cold isosmolar MgCl_2 (ref. 14) or NaSCN (ref. 15) solutions to remove 'extracellular' tracer.

Provided that extracellular tracer had exchanged with less than 20% of the intracellular cation, the uptake of ^{24}Na or ^{42}K was usually linear with time except for a small deviation

at times less than about 20 min. This initial curvature probably represents the penetration of tracer into a small space (less than about 1.5%) beyond that accessible to ^{131}I -albumin yet still extracellular. Fluxes (meq per l cells h^{-1}) of sodium and potassium were calculated from the ratio of the slope of the regression line fitted to the uptake of the tracer and the extracellular specific activity of the cation.

The temperature dependence of the cation fluxes is illustrated by the Arrhenius plots in Fig. 1. It was surprising to find that the sodium flux was maximal at about 22° C at which temperature the flux was three times greater than at 38° C. By contrast, the potassium flux decreased as the temperature was lowered from 38° C to about 12° C, and then passed through a minimum. At all temperatures the cells remained at constant volume throughout the uptake of the tracers as indicated by the measurements of intracellular haemoglobin. Within experimental error the intracellular cation contents did not vary significantly with temperature, although cell volume at 18–22° C was slightly ($\leq 3.7\%$) greater than at 38° C. This difference in cell volume, however, could not account for the results as it is established that as dog red cells swell sodium flux decreases and potassium flux increases^{16–18}.

Vieira *et al.*¹⁹ have shown that the apparent activation energies for both the hydraulic conductivity and the diffusion permeability of water in dog red cells are constant from 37° C to 7° C. Taken in conjunction with the present results, this implies that the translocation of water, potassium and sodium across the membrane of this type of cell takes place by means of independent mechanisms and that the transport of either cation cannot occur through water-filled pores in the membrane. Furthermore, the lack of coupling between the effects of temperature on the transport of the different species makes it unlikely that any long-range reorganisation of the membrane lipids occurs when the fluxes of sodium and potassium are maximal and minimal respectively. It seems more likely that changes in the conformation of localised lipid-protein complexes are involved.

Davson and Reiner²⁰ first showed that the net efflux of sodium from cat red cells suspended in KCl solutions was maximal at around physiological temperatures and, more recently, this has been confirmed in cells under steady-state conditions²¹. Therefore, while cat and dog cells have a number of characteristics in common in that they are sodium-rich, have a low Na-K ATPase activity²² and have a cation transport system that is dependent on cell volume^{15–21,23}, it would seem that there is a marked shift in the temperature at which the permeability of the membranes to sodium is maximum. It may be possible to relate this to the small but definite differences in the lipid composition of the membranes²⁴.

It is worth noting perhaps that a 'suggestion' of a phase-transition at about 22° C in human red cell ghosts has been found recently using a dilatometric technique²⁵.

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Deficiency of two enzymes of galactose metabolism in kangaroos

THE starting point of the investigation reported here was the observation by one of us of diarrhoea and occasional cataract formation in bottle-fed orphan pouch-young kangaroos. As kangaroo milk has a very low lactose content¹⁻³ and the intestinal lactase content of pouch-young kangaroo is also low⁴, we thought that these marsupials may not utilise galactose, present mainly as polygalactan in milk, by the usual pathway involving galactokinase (E.C. 2.7.1.6) and galactose-1-phosphate uridyl transferase (E.C. 2.7.7.12) (transferase). With this postulate, and the role of deficiency of these two enzymes in human cataractogenesis in mind, galactokinase and transferase were assayed in haemolysates from the grey (*Macropus giganteus*) and the red kangaroo (*Megaleia rufa*).

Blood was collected from lateral tail veins of unanaesthetised adult male and female kangaroos, and by venepuncture from normal adult humans of both sexes. Haemolysates were pre-

TABLE 1 Erythrocytic galactokinase and galactose-1-phosphate uridyl transferase of grey and red kangaroos

Species	Galactokinase (μmol galactose phosphorylated $\text{h}^{-1} \text{ml}^{-1}$ RBC)		Galactose-1-phosphate uridyl transferase (U g^{-1} haemoglobin)	
	No.	Mean $\pm$ s.d.	No.	Mean $\pm$ s.d.
Grey kangaroo	22	0.039 ± 0.013	19	2.2 ± 1.7
Red kangaroo	21	0.033 ± 0.009	20	10.6 ± 3.5
Human	200	0.213 ± 0.028 (7)	61	21.4 ± 3.2

Statistically, galactokinase and transferase values from both species of kangaroos were significantly different ($P < 0.001$ in all comparisons) from human values. Galactokinase was not significantly different between two species of kangaroos, but transferase was so ($P < 0.001$). RBC, red blood cells.

TABLE 2 Amount of methaemoglobin reduced in 3 h with either galactose or glucose as substrate by erythrocytes from grey and red kangaroos

Species	No.	% Methaemoglobin reduced	
		Galactose substrate	Glucose substrate
Red kangaroo	6	5.0 ± 1.8	15.2 ± 5.3
Grey kangaroo	6	1.9 ± 1.4	11.4 ± 3.6
* Human	14	10.2 ± 2.0	28.0 ± 5.2

* Human values as reported earlier⁵.

pared and galactokinase was assayed as previously described⁵. Transferase was determined using Sigma Kit (Sigma), by the UDPG consumption test⁶, as described in *Sigma Technical Bulletin* No. 600-UV. A normal blood sample was always used as control for each batch of assays for both enzymes. To study the overall utilisation of galactose by erythrocytes, a modified methaemoglobin reduction test was performed for 3 h as described earlier⁵, with galactose as substrate; glucose was used as substrate for comparison.

The results of enzyme assay are shown in Table 1. In both red and grey kangaroos, galactokinase activity was about one-sixth of the normal human value. It should be noted that red cell galactokinase activity in both species was much lower than in various other mammals⁵. A distinct difference between the two species was observed with regard to transferase, in that the grey kangaroo almost completely lacked this enzyme, whereas the red kangaroos had levels approximating those in humans heterozygous for galactose-1-phosphate uridyl transferase deficiency. Erythrocytic haemolysates from seven of the nineteen grey kangaroos had enzyme activity of less than 1U, and four of these had no activity at all. In both species, similar values of galactokinase and galactose-1-phosphate uridyl transferase were found in males and females.

The combined effect of the deficiency of both galactokinase and transferase in grey kangaroos and of galactokinase, but not transferase, in red kangaroos is suitably demonstrated by measuring rates of methaemoglobin reduction with galactose as substrate (Table 2). While both grey and red kangaroos were substantially less able to reduce methaemoglobin than humans, this deficiency was more pronounced in grey than in red kangaroos. The faster rates of methaemoglobin reduction with glucose as substrate indicate that the galactose pathway limits the rate of methaemoglobin reduction in both kangaroos and humans.

We are presenting these results to draw attention to the possibility that other marsupials may prove to be deficient in enzymes of galactose metabolism, and that such animals may provide useful models for galactose cataractogenesis. Mathai *et al.*⁷ investigated the possibility of using California sea lions (*Zalophus californianus*), whose milk is completely deficient in lactose, for the study of the pathogenesis of galactosaemia in man, but found that their erythrocytes were not deficient in either galactokinase or transferase activity, possibly due to the high galactose content of mucopolysaccharides in various aquatic organisms on which they feed. In view of their almost complete lack of transferase, grey kangaroos may be a very useful animal model for the study of galactosaemia in man.

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Mammatropic effect of prolactin enhanced by thyroidectomy

THE role of thyroid hormones in relation to mammary gland structure and function has received sporadic attention. It has been shown in the rat that thyroid hormones are not necessary for mammary development¹ and that in ovariectomised, adrenalectomised, hypophysectomised rats, prolactin and growth hormone are the principal hormones responsible for lobulo-alveolar growth². The slight stimulatory effect of thyroid hormones on mammary growth and lactation in animals has been attributed to their general metabolic effects^{3,4}. There are, however, a few contrary reports in the literature describing greater mammary development in rats rendered hypothyroid^{5,6}, which have remained unexplained. The present study was undertaken to clarify the relationship between thyroid hormones and mammary growth, with special reference to prolactin. Interest in this field was primarily aroused by several clinical and epidemiological reports associating thyroid hypofunction with breast cancer^{7,8}.

Two separate experiments were carried out. In the first, thirty-six Sprague-Dawley female rats, weighing about 200 g, were divided into six equal groups. They were fed on a commercial diet and given tap water *ad libitum*. All rats, including controls, were given oestradiol (8 µg subcutaneously s.c.) daily from day 1 of the experiment to day 10 inclusive⁹. In addition, rats in each group were treated as described in the legend to Fig. 1. The animals were killed on day 16. The left inguinal mammary glands were removed, fixed in buffered formal saline, processed for histology, and the sections stained with haematoxylin and eosin (Fig. 1). In oestrogen-primed rats, thyroidectomy induced a marked stimulation of mammary development, which was completely suppressed by replacement doses of thyroxine. A marked suppression of mammary stimulation also occurred when 2-bromo- α -ergocryptine (CB154), a compound known to reduce but not abolish prolactin secretion⁹, was administered. By raising the prolactin level with perphenazine¹⁰, the mammary glands of thyroidectomised rats could be still further stimulated with the production of macroscopic secretion.

In the second experiment twenty Sprague-Dawley rats, weighing about 200 g, were divided into two groups of ten and fed and watered as in the first experiment. All rats were ovariectomised and thyroidectomised on day 1 of the experiment. Each rat received daily injections of oestradiol (1 µg s.c.), progesterone (3 mg s.c.) and thyroxine (2 µg per 100 g body weight i.p.) from day 1 to day 15. One group received bovine thyrotropic hormone daily (TSH:1.5 IU (s.c.)) from day 11 to day 15, the second group remained as untreated controls. All rats were killed on day 16 and the mammary glands removed and processed as in the first experiment. Histological examination showed no difference between the two groups.

The marked enhancement of mammary development by thyroidectomy in the first experiment might be explained

by the following possibilities: (1) thyroidectomy causes an increase in the secretion of prolactin and/or growth hormone; (2) thyroidectomy causes an increased secretion of ovarian oestrogens which in turn stimulates prolactin secretion; (3) TSH, which is increased after thyroidectomy, has mammatropic properties; (4) prolactin exerts a greater mammatropic effect in the absence of thyroid hormones.

The first two possibilities are unlikely as measurement of prolactin and growth hormone levels in the blood of hypothyroid rats, by sensitive radioimmunoassay methods, has shown that there is no significant change in the prolactin level, whereas there is a dramatic fall in the level of growth hormone¹¹. Previous observations by Leonard and Reece⁵ of greater mammary development in thyroidectomised-ovariectomised rats compared to ovariectomised controls, also excludes the involvement of ovarian hormones in this phenomenon. The third possibility that TSH may have mammatropic properties is proved unlikely by the second experiment. Therefore, it seems that the fourth possibility is the most likely: that in the absence of thyroid hormones, the mammatropic effect of a normal level of endogenous prolactin is markedly increased and that further stimulation occurs when the prolactin level is raised by pharmacological means.

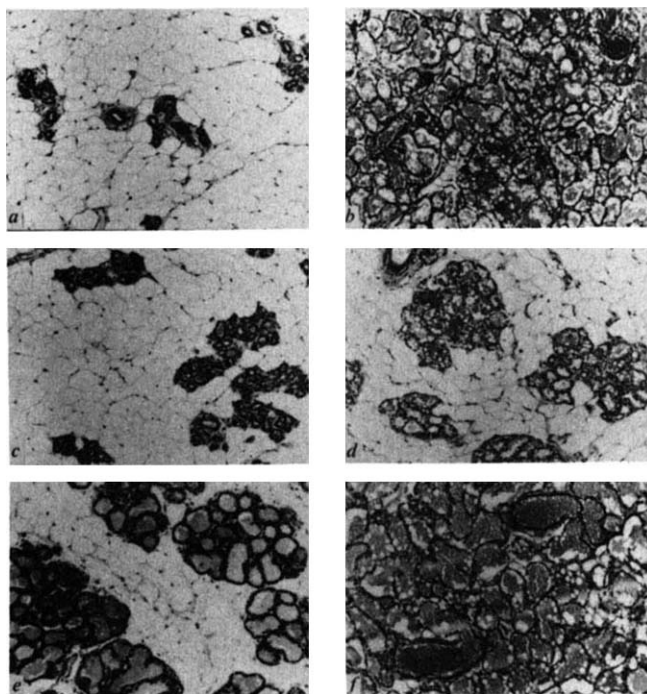


FIG. 1 Photomicrographs of sections of mammary gland showing the relationship between the effects of prolactin and thyroxine on mammary development in the rat. Stain: haematoxylin and eosin $\times 52$. All rats, six per group, were given oestradiol 8 µg s.c. daily from day 1 to day 10 inclusive and killed on day 16. In addition, rats in each group were treated as indicated: a, Control group (no additional treatment) showing clusters of ducts amidst mammary fat with little alveolar formation. b, Thyroidectomy (on day 1), marked duct and alveolar development replacing mammary fat. Alveoli contain secretion. c, Thyroidectomy (on day 1) + thyroxine (in alkaline solution) 2 µg per 100 g body weight per day i.p. from day 1 to day 15 mammary development comparable with the control group. d, Thyroidectomy (on day 1) + 2-bromo- α -ergocryptine (in 70% ethanol) 400 µg per 100 g body weight per day i.p. from day 1 to day 15. Reduction in the quantity of developed glandular tissue compared with b occupying a lesser volume of mammary fat. e, Perphenazine 5 mg per kg body weight per day s.c. from day 11 to day 15. Marked glandular development with alveolar secretion. f, Thyroidectomy (on day 1) + perphenazine 5 mg per kg body weight s.c. from day 11 to day 15. Extreme mammary development, alveoli distended with secretion. Total replacement of mammary fat.

If one assumes that the converse holds true, that excess thyroxine inhibits the mammatropic action of prolactin, then the phenomenon reported by Meites and Kragt¹² might be explained in the light of my findings. These workers observed prolactin induced mammary development around an ectopically transplanted pituitary in immature hypophysectomised rats, which was unexpectedly abolished when thyroxine was administered, despite an increase in weight of the animals. Although thyroid hormones are galactopoietic in physiological doses¹³, suppression of lactation is known to occur in the rat¹⁴ and mink¹⁵ when pharmacological levels of thyroactive substances are administered; large amounts of thyroid hormones being required presumably to block the elevated prolactin level during lactation.

A prolactin-thyroxine antagonism is also known to occur in some lower vertebrates. In urodele development, thyroxine is responsible for 'land drive' and prolactin for 'water drive', the effect of one hormone being reversed by the other under experimental conditions¹⁶. Etkin has recently drawn attention to a similar antagonism in amphibian metamorphosis¹⁷; repeated injections of prolactin in larval tadpoles prevents thyroxine-induced metamorphosis into adult structures until additional thyroxine is given¹⁸. Bern and Nicoll¹⁹ have suggested that this antagonism takes place at a peripheral tissue level. The present experiment indicates a similar antagonism at the level of the rat mammary epithelium.

If hypothyroidism sensitises the rat mammary epithelium to the action of prolactin, this condition should also enhance 7,12-dimethyl-benz(a)anthracene (DMBA)-induced mammary tumorigenesis, which is prolactin dependent²⁰. Shellabarger²¹ obtained twice as many tumours in rats rendered hypothyroid by propylthiouracil (PTU) administered for only two weeks before DMBA, compared with controls; but continuous PTU treatment, maintained after DMBA, suppressed tumour production. Therefore, hypothyroidism enhances tumour initiation but cannot promote tumour growth, presumably due to disappearance of growth hormone from blood²¹.

The antagonism between prolactin and thyroxine at the level of the rat mammary gland is a new finding the mechanism of which remains to be investigated. In view of a similar observation in some lower vertebrates, a wider implication of this phenomenon cannot be excluded. It is possible that the level of thyroid hormones in blood determines the 'set point' for the mammatropic effect of prolactin. Reports of massive virginal breast hypertrophy in association with hypothyroidism²² and objective and subjective improvement after thyroid therapy in women suffering from mammary dysplasia^{23,24} point to possible clinical evidence of a similar phenomenon.

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Oestradiol inhibition of collagenase role in uterine involution

THE uterus of the rat provides an ideal organ for the study of collagen breakdown; during the *post partum* involution of this organ the half-life of the uterine collagen is reduced to 24–30 h. Previous studies have shown that this rapid breakdown of collagen in the rat uterus is accompanied by extensive phagocytosis of collagen^{1–3}, elevated lysosomal enzyme levels⁴, the presence of hydroxyproline in lysosome-like particulates, and an increase in the concentration of a collagenolytic cathepsin which digests collagen at acid pH (ref. 5). All of these findings are consistent with the intracellular ingestion and digestion of the extracellular collagen fibres.

None of these findings, however, gives a clear explanation of how the fibres are broken into fragments and brought inside the cell, nor do they allow an evaluation of the relative contributions of intra and extracellular digestion processes. It has been suggested that an extracellular collagenase active at neutral pH is required for the first step in collagen breakdown⁶. Such a collagenase has been demonstrated by culturing fragments of rat uterus; the collagenase is secreted into the culture medium from which it can be purified and characterised⁷. Enzyme is produced only by tissue from involuting uteri⁶. This method enables measurement of the potential of the tissue for collagenase elaboration but it does not reveal how much enzyme is actually present in the tissue. Recently, we have developed a method which enables the direct measurement of collagenase in the particulate fraction of uterine homogenates⁸. We have shown that the method is relatively specific for collagenase⁹ and that the enzyme being measured is a true collagenase¹⁰. Here we report findings obtained by applying this assay to the rat uterus in various stages of pregnancy and involution.

It has further been shown that oestradiol is a potent and selective inhibitor of collagen breakdown in the uterus¹¹. In searching for explanations for this effect it was shown that lysosomal enzyme levels, acid collagenolytic cathepsin activity, and collagen peptidase activity remained unaffected⁵. It has also been shown that oestradiol does not affect collagenase production by uterine slices in culture¹². We investigated whether oestradiol affected uterine collagenase when the hormone was administered to the intact rat.

TABLE 1 Collagenase activity in the involuting uteri of control and estradiol-treated rats

Experimental group	No.	Hydroxyproline (mg per uterus)	Collagen digestion (%)	Collagen digestion (μ g hydroxyproline per uterus)
Non gravid	5	1.36 $\pm$ 0.05	2.0 $\pm$ 1.0	27 $\pm$ 14
Pre partum (1 d)	5	6.89 $\pm$ 0.90	0.70 $\pm$ 0.68*	49 $\pm$ 48
Parturition	6	8.80 $\pm$ 2.20	2.9 $\pm$ 1.1	226 $\pm$ 113
Post partum controls:				
Day 1	5	8.10 $\pm$ 1.39	5.1 $\pm$ 2.3	396 $\pm$ 129
Day 2	8	4.63 $\pm$ 0.39	14.0 $\pm$ 2.8	658 $\pm$ 182
Day 3	6	1.73 $\pm$ 0.37	12.9 $\pm$ 2.9	220 $\pm$ 52
Day 4	7	0.89 $\pm$ 0.35	8.2 $\pm$ 1.8	77 $\pm$ 36
Oestradiol-treated:				
Day 1	5	8.08 $\pm$ 1.32	4.7 $\pm$ 1.4	368 $\pm$ 84
Day 2	8	4.88 $\pm$ 0.49	4.6 $\pm$ 0.9†	237 $\pm$ 120†
Day 3	6	3.94 $\pm$ 0.34†	5.3 $\pm$ 0.8†	207 $\pm$ 30
Day 4	5	3.80 $\pm$ 0.89†	2.1 $\pm$ 1.0†	73 $\pm$ 20

All values given as mean $\pm$ s.d.

* This value differs from parturition by $P < 0.01$ and from non-gravid by $P < 0.05$.

† Value significantly ($P < 0.001$) less than relevant control.

Pregnant female rats were purchased from Sprague-Dawley Farms, Madison, Wisconsin, at the 17th day of gestation and were maintained on Purina rat chow. The time of delivery was recorded and the young were allowed to nurse. Injections of 17β -estradiol (Mann Research Laboratories, New York) were administered intraperitoneally as a suspension of the hormone in 0.9% NaCl (100 μ g in 0.1 ml.) shortly after delivery was complete and on each successive day. Uteri were removed and dissected free of mesentery and placental sites. Preparation of tissue homogenates and determination of collagenase activity in the 6000g pellets were as previously described⁸. The collagen of the uterus served as the substrate for the collagenase, and EDTA blanks were used in every case.

The major findings are presented in Table 1. The % collagen digestion was measured in the 6000g pellet from 0.2 g wet uterine tissue in a total volume of 2.0 ml. All of the pellets at all points contained approximately the same amount of collagen (about 700 μ g hydroxyproline) with the exception of the 3 and 4 d controls (about 500 μ g). Therefore, as substrate and tissue weight were constant, the results are essentially a measure of enzyme concentration. The total digestion per uterus (final column) was calculated by multiplying the % digestion by the total hydroxyproline content for each sample. This provides an estimate of the total units of enzyme per whole organ.

Very little collagenase activity can be found in non-gravid uteri and in uteri 1 d before parturition. Collagenase rises by the time of parturition and then rapidly increases to a peak of concentration at day 2–3 and of total units at day 2 post partum. By 4 d, involution is almost complete; there is enough enzyme to digest 8% of the collagen, but the uterus contains very little remaining collagen. These data on collagenase activity correlate closely with previous studies on the rate of collagen breakdown in the involuting uterus¹¹. The maximum rate of hydroxyproline loss occurs between day 1 and 3 (Table 1). The data also agree with the finding of Jeffrey *et al.*⁶ that maximum production of collagenase in cultures of uterine tissue is observed with tissue removed at 1–2 d post partum. The correlation of activity with rate of collagen loss is not perfect, because at parturition there is an appreciable level of collagenase activity but almost no collagen degradation for the first 24 h. It should also be noted that the digestion of collagen during the 20 h assay is only about one-fifth that which occurs *in vivo* during the same period (such as day 2). The system *in vitro*, however, has been diluted 10-fold and optimum conditions may not obtain. On the other hand, other pathways for collagen breakdown may also be operative *in vivo*.

Oestradiol treatment strongly inhibits collagen breakdown. The values of hydroxyproline per uterus show that the main inhibitory effect occurs between day 2 and 3 (Table 1). It is just at the beginning of this period that the collagenase levels of oestradiol-treated animals vary most markedly from controls. At day 2 the oestradiol-treated tissue has only about one-third the control value of collagenase, whether expressed as concentration or as total units per uterus. The collagenase content never increases over that found at day 1; the concentration continues to remain low through day 4. Whereas the total activity per uterus approaches control values on days 3 and 4, the low enzyme concentration and the high collagen content prevent the treated uteri from maintaining a normal rate of collagen breakdown.

Oestradiol does not act by altering the collagen substrate: solubility and susceptibility to added uterine collagenase, acid collagenolytic cathepsin, and trypsin are unaffected. Uterine homogenates contain inhibitors which are normally discarded with the supernatant before the assay. Figure 1 shows that the supernatants from oestrogen-treated rats are, if anything, even less inhibitory than the control supernatants. Moreover, a study of the supernatants by the method of Sakamoto *et al.*¹³ indicates that very little collagenase is present. All evidence

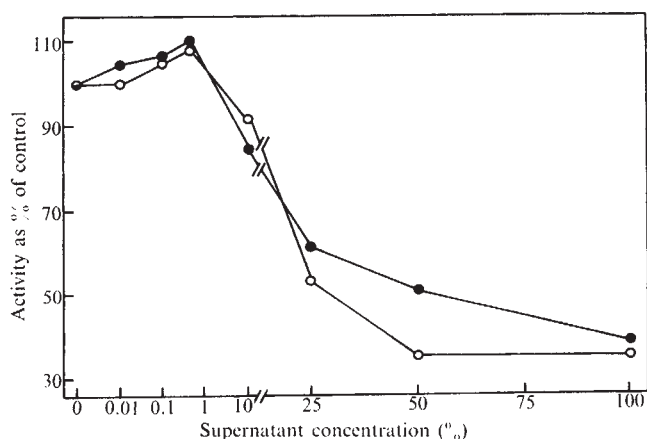


FIG. 1. Effect of 6000g supernatants of uterine homogenates on collagenase activity in uterine pellets. The preparation of supernatants and pellets and the enzyme assays are as previously described⁸. The supernatants were adjusted to pH 7.6 and added to control pellets to produce the final concentration shown. ○, Supernatant from 2 d post partum uterus of control rat; ●, supernatant from corresponding uterus of oestradiol-treated rat. A digestion of 100% of the control value corresponds to 15.2% digestion of the collagen.

to date points to the presence of fewer active molecules of collagenase in the oestrogen-treated uteri.

A recent report by Jeffrey *et al.*¹² showed that progesterone added to cultures of rat uterine tissue inhibits collagenase production, whereas oestradiol has no effect. This suggests that the oestradiol effects observed in the intact rat may not be directly mediated by the uterus.

It has been shown by Yoshinaga *et al.*¹⁴ that the rat ovaries at the time of parturition produce almost 1 μg of oestrogen per day. We showed earlier¹¹ that 1 μg of oestradiol per day clearly inhibited collagen breakdown so that by 4 d the treated uteri contained twice as much collagen as the controls. The present dose of 100 μg was selected to maximise this effect, leaving four times as much collagen at 4 d.

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Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone

THYROTROPIN-RELEASING hormone (TRH) (pyroglutamyl-histidyl-proline-amide) has been reported to ameliorate mental depression^{1,2}. The mechanism of action of the tripeptide in this condition is unknown. It may be connected with changes in the activity of noradrenergic or 5-hydroxytryptaminergic neurons which are thought to be involved in depressive states^{3,4}. Therefore, we have investigated the action of TRH on the turnover of biogenic amines in rat brain.

Male albino rats of 200 g, Wistar origin, were injected intraperitoneally with TRH. Thereafter noradrenaline, dopamine, 5-hydroxytryptamine (5-HT) as well as their corresponding main metabolites 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG, isolated as the sulphate ester),

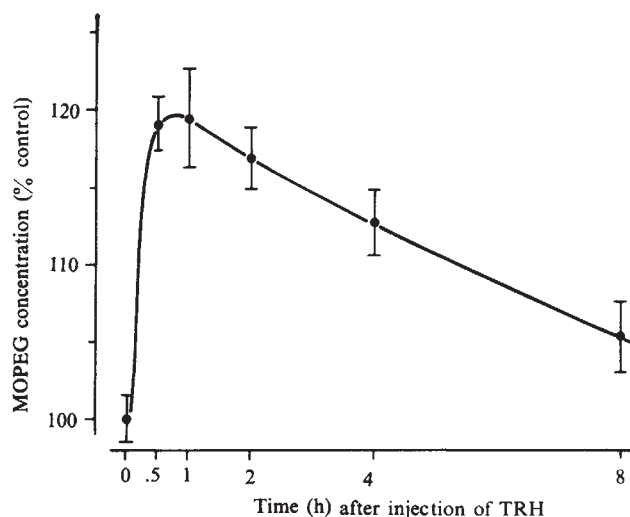


Fig. 1 Effect of intraperitoneal injection of 10 mg kg⁻¹ TRH on the content of 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG) in the cerebral cortex of rats. Each value is expressed in percent of untreated controls (95.7 $\pm$ 2.9 ng g⁻¹ = 100%) and represents the average with s.e.m. of results from twenty to forty determinations per group performed in four to eight experiments. Each determination was carried out in two pooled cerebral cortices. Significance against controls: MOPEG concentration 0.5, 1, 2 and 4 h after TRH injection. $P < 0.001$ (Student's *t* test).

homovanillic acid and 5-hydroxyindoleacetic acid were measured in whole brain and/or various brain parts⁵ by spectrophotofluorimetric methods⁶⁻¹⁰. In other experiments the action of triiodothyronine (T₃) on the levels of MOPEG in different brain regions was determined. In animals thyroidectomised 2 d before TRH injection, estimations of MOPEG were carried out in the same cerebral regions. In addition, the effect of TRH on the accumulation of ¹⁴C-noradrenaline and ¹⁴C-dopamine in the whole brain was measured in rats injected with L-3-¹⁴C-tyrosine (30 μg in 20 μl saline; specific activity 53.9 mCi mmol⁻¹) through a permanent cannula implanted in one lateral cerebral ventricle¹¹. Labelled noradrenaline and dopamine were isolated by adsorption on alumina, separated by column chromatography on Dowex 50 $\times$ 8 and estimated in a liquid scintillation counter^{12,13}. Normal and thyroidectomised rats not injected with TRH served as controls. In the thyroidectomised animals the concentration of total thyroxine in the blood serum (measured by competitive protein-binding analysis¹⁴) had dropped to about one-fifth of its normal value and did not rise following TRH injection.

Two hours after administration of 10 mg kg⁻¹ TRH to normal rats, the cerebral content of noradrenaline, dopamine, 5-HT, homovanillic acid and 5-hydroxyindoleacetic acid did not significantly differ ($P > 0.05$) from that in untreated controls (% values after TRH: 101.0 $\pm$ 1.5%, 99.3 $\pm$ 2.9%, 102.0 $\pm$ 2.5%, 104.0 $\pm$ 3.2% and 98.7 $\pm$ 3.2%, respectively; controls: 100%). The concentration of MOPEG was, however, significantly increased ($P < 0.01$) in the whole brain as well as in the various brain regions (Table 1). In the cerebral cortex the action of TRH lasted for at least 4 h, maximal values of MOPEG (about 120% of controls) being reached between 30 min and 1 h after injection (Fig. 1). The concentration of MOPEG in various brain parts of thyroidectomised rats did not differ from that seen in controls with the exception of mesencephalon plus medulla oblongata where MOPEG concentrations were significantly increased after thyroidectomy. This elevation could not be explained. Injection of TRH, however, caused a rise of MOPEG concentration in all brain regions of thyroidectomised rats similar to that occur-

TABLE 1 Effect of TRH and T₃, injected intraperitoneally, on the content of 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG) of whole brain and (or) various parts of rat brain

	Normal		Thyroidectomised	
	Controls	With TRH	Controls	With TRH
Whole brain	121.3 ± 6.0	156.1 ± 9.0		
Cortex	105.2 ± 3.1	126.4 ± 4.2	93.5 ± 2.2	103.1 ± 2.5
Hypothalamus	182.7 ± 11.0	226.3 ± 8.3	150.8 ± 5.8	167.6 ± 5.6*
Mesencephalon plus medulla oblongata	115.3 ± 4.9	143.5 ± 6.0	117.0 ± 3.9	127.0 ± 3.7†
Rest of brain	103.0 ± 2.0	128.0 ± 5.5	105.0 ± 3.9	128.0 ± 4.3
			147.3 ± 6.1	119.3 ± 4.6
			204.6 ± 8.2	175.4 ± 6.4

Measurements were carried out 2 h after i.p. administration of 10 mg kg⁻¹ TRH or 20 µg kg⁻¹ T₃, respectively. Thyroidectomy was performed 2 d before injection of TRH. The figures indicate ng g⁻¹ and represent means with s.e.m. of values obtained from twelve to twenty-eight determinations per group performed in three to seven experiments. Each determination was carried out with one single whole brain or with pools of two to three brain parts.

Significance (compared with corresponding controls).

* $P < 0.05$.

† $P > 0.05$; all other values, $P < 0.01$ (Student's *t* test).

ring in non-operated animals (Table 1). T₃ (20 µg kg⁻¹) administered intraperitoneally (i.p.) to normal rats caused a rise of MOPEG in cerebral cortex, hypothalamus and mesencephalon plus medulla oblongata which was less pronounced than that due to TRH. In the 'rest of the brain' a similar increase was found after T₃ and TRH administration.

Since TRH did not change rectal temperature, the TRH-induced increase of cerebral MOPEG was probably not due to hypothermia in which noradrenaline turnover is accelerated¹⁵. An inhibition of the clearance of MOPEG from the brain is also unlikely, since other amine metabolites (homovanillic acid and 5-hydroxyindoleacetic acid), which are thought to be cleared by a similar transport mechanism as MOPEG¹⁶, were not affected by TRH. Therefore, the TRH-induced increase of MOPEG in the brain is rather due to an enhanced release of noradrenaline with consecutive catabolism of the amine to the glycol. This is confirmed by histofluorimetric experiments. Thus, the α -methyl-*p*-tyrosine-induced diminution of the green fluorescence specific for catecholamines was accentuated by TRH in the hypothalamus and the cerebral cortex which mainly contain noradrenergic fibres (Constantinidis *et al.*, in preparation).

Intraperitoneal injection of 10 mg kg⁻¹ TRH 1 h before intraventricular administration of L-3-¹⁴C-tyrosine enhanced the accumulation of ¹⁴C-noradrenaline formed in the whole brain within 1 h (controls: 1,393 ± 55 c.p.m. g⁻¹, TRH-treated: 1,600 ± 62 c.p.m. g⁻¹; $P < 0.02$, $n = 25$). This indicates an increased synthesis of cerebral noradrenaline which probably compensated for its enhanced release since the cerebral content of the endogenous amine remained unchanged. On the other hand, TRH did not influence the accumulation of ¹⁴C-dopamine in the brain (controls: 4,291 ± 198 c.p.m. g⁻¹; TRH: 4,433 ± 296 c.p.m. g⁻¹; $P > 0.05$, $n = 15$) in agreement with the fact that the hormone has no effect on endogenous homovanillic acid (see above). These findings indicate that TRH enhances the turnover of noradrenaline in the rat brain. The potentiation by TRH of the behavioural changes caused by L-dopa plus pargyline in hypophysectomised mice¹⁷ might be connected with this effect. Since TRH also acted in thyroidectomised rats, a mediation by the hypophyseothyroid axis does not seem to be a major mechanism in the acceleration of noradrenaline turnover. Nevertheless, as indicated in the experiments with T₃, a contributing role of this hormone in normal animals cannot be excluded.

In conclusion, TRH probably causes an activation of noradrenergic neurons in the brain. This effect might be connected with the antidepressant action of the tripeptide in man and if so, support the hypothesis of an involvement of noradrenaline in mental depressive states.

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Multiquantal release of acetylcholine in mammalian ganglia

IN the course of studies on synaptic transmission in the hypogastric ganglia of male guinea pigs it became apparent that in many ganglion cells repetitive stimulation of the hypogastric nerve at 1-Hz raised the frequency of spontaneous synaptic potentials from less than 1 min⁻¹ to about 1 s⁻¹. Many of these cells had only a single preganglionic fibre synapsing with them so that the spontaneous synaptic potentials arising from a single nerve could be readily observed. Blackman *et al.*¹ described large spontaneous synaptic potentials in this preparation and we decided to examine the amplitude distributions of spontaneous synaptic potentials

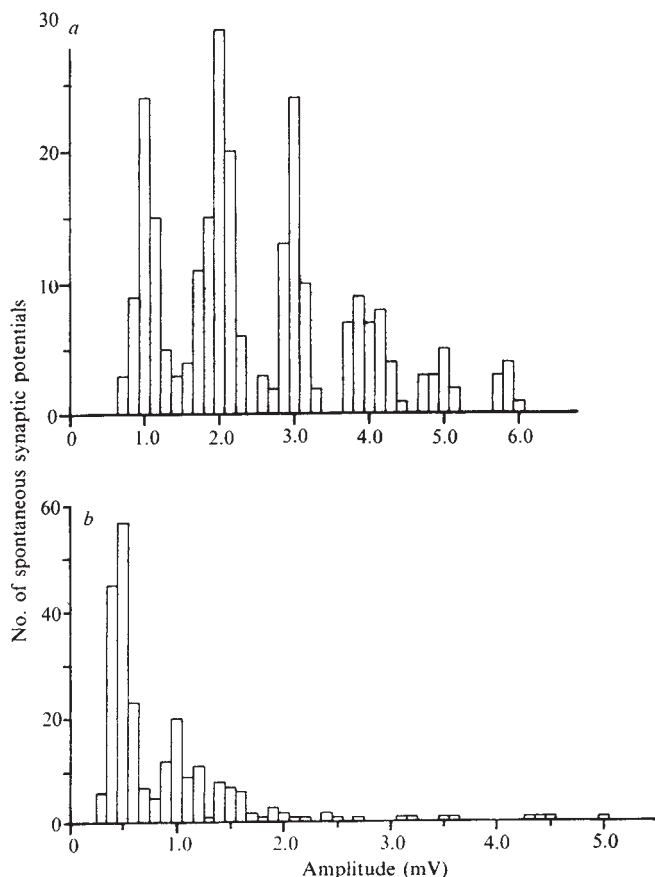


FIG. 1 Amplitude distributions of spontaneous synaptic potentials in *a*, a cell whose single input was repetitively stimulated at 1 Hz; *b*, a single input cell in 15 mM K^+ .

recorded during repetitive stimulation (1 Hz). Although 'spontaneous' synaptic potentials which were not time locked to the stimulus occurred occasionally during the evoked response, only those recorded after the membrane potential had returned to normal were selected for analysis.

A typical amplitude distribution for the spontaneous synaptic potentials recorded from a cell during prolonged repetitive stimulation (at 1 Hz) of its single input can be seen in Fig. 1*a*. The most striking feature is the appearance of a number of distinct groups in the distribution; if each group was treated as a separate distribution, means of $1.1(\pm 0.02, \text{S.e.})$, $2.0(\pm 0.02)$, $3.0(\pm 0.02)$, $4.0(\pm 0.03)$, $4.9(\pm 0.04)$ and $5.8(\pm 0.03)$ mV were obtained. This phenomenon was observed in every cell (seven cells with a single input) from which spontaneous synaptic potentials were recorded during repetitive stimulation. To determine whether the observations were due to some effect of stimulation, another method of raising the frequency of spontaneous synaptic potentials was used.

Blackman *et al.*² found that increasing the K^+ concentration in the bathing medium markedly increased the frequency of spontaneous synaptic potentials recorded from frog sympathetic ganglion cells. In the present experiments it was found that increasing the K^+ concentration from 5 mM to 15 mM raised the frequency of spontaneous synaptic potentials to about 1 s^{-1} even if the hypogastric nerve was not stimulated. Thus a large sample of spontaneous synaptic potentials could be recorded in a relatively short time. If the hypogastric nerve was stimulated the number of preganglionic inputs on the cell could still be determined. Figure 1*b* shows the amplitude distribution obtained in 15 mM K^+ from a cell with a single input. Several distinct groups can be seen, although samples of larger amplitudes were too small to allow analysis. If the groups were treated as separate dis-

tributions the means obtained were $0.5(\pm 0.008)$ mV, $1.0(\pm 0.02)$ mV, $1.5(\pm 0.02)$ mV, $2.0(\pm 0.06)$ mV, $2.4(\pm 0.06)$ mV.

This preparation clearly differs from the rat diaphragm, where Hubbard and Jones³ found that the amplitude distribution of spontaneous endplate potentials, although often skewed to higher amplitudes, showed no evidence of grouping about multiples of the modal amplitude. They concluded that the 'giant' spontaneous endplate potentials first described by Liley^{4,5} were due to larger than normal quanta. The 'giant' spontaneous endplate potentials at the rat neuromuscular junction however, have a different time course to the miniature endplate potentials⁶ and their frequency is unaffected by raised K^+ concentration or repetitive stimulation⁵.

The spontaneous synaptic potentials recorded in the hypogastric ganglia fit a multimodal amplitude distribution. There are at least two possible explanations of these results. (1) The quanta (or packets) of acetylcholine (ACh) stored in the preganglionic nerve terminals may be of a number of different sizes; or (2) spontaneous release of ACh may occur in multiquantal units.

The first explanation implies that the contents of the larger quanta would be approximately integral multiples of the contents of the smallest quanta. If a vesicle represents a quantum and the concentration of ACh in the vesicle is approximately uniform throughout the population of vesicles then their diameters should conform with the series 1, 1.26, 1.44, 1.59, 1.71 and soon with the diameter of the smallest vesicle taken as 1 unit. Any attempt to find evidence for a distribution of vesicle diameters with peaks at these relative values would be difficult as such measurements would be complicated by the uneven sectioning of vesicles inevitable in electron-microscopy. A quantitative study of the diameter and density of vesicles in the nerve terminals in the hypogastric ganglia would be necessary to completely eliminate this hypothesis.

Multiquantal spontaneous synaptic potentials have been described in the chick ciliary ganglion⁷ and in the parasympathetic ganglion cells of the septum of frog heart⁸. Martin and Pilar⁷ proposed that the spontaneous release of a single quantum created a small but finite probability that a second quantum would be released virtually simultaneously, the 'drag' hypothesis. They were able to fit the amplitude distribution of spontaneous synaptic potentials to a Poisson distribution by calling a unit response a failure and a double response a single and calculating a 'quantal content' of the 'dragged', synaptic potential. In the present experiments, however, no good fit could be obtained to either a Poisson or a binomial distribution using the 'drag' hypothesis. Dennis *et al.*⁸ were able to fit their data with a model based on the availability of release sites; however, their analysis could not be applied to the present experiments as no evidence was available to the maximum number of quanta which could be spontaneously released.

The quantum contents of subthreshold evoked synaptic potentials in most cells of the superior cervical ganglion⁹ and the sympathetic chain ganglia¹⁰ of guinea pigs do not appear to fit a Poisson distribution. In these preparations large spontaneous synaptic potentials can be observed and it is possible that the mechanism responsible for the large spontaneous synaptic potentials is also involved in the evoked release. Large increases in the frequency of spontaneous synaptic potentials due to repetitive preganglionic stimulation have, so far, only been observed in cells with 'strong fibre' type input¹; such input always initiates an action potential obscuring the evoked synaptic potential. By raising the $Mg^{2+} : Ca^{2+}$ ratio in the bathing solution the amount of ACh released by a single impulse can be reduced and the quantal content of individual synaptic potentials could then be measured. Altering the $Mg^{2+} : Ca^{2+}$ ratio, however, may

change the amplitude distribution of the spontaneous synaptic potentials⁸ and an examination of the ionic basis of both the spontaneous and the evoked release mechanisms will be necessary to complete this analysis.

The occurrence of large spontaneous synaptic potentials during low frequency repetitive stimulation could be of significance in the function of autonomic ganglia. Most autonomic ganglia receive tonic synaptic input from the central nervous system, *in vivo*¹¹, or from neurones in the periphery¹² and the cells in these ganglia receive input from many preganglionic nerve fibres¹³⁻¹⁵. In most cells, summation of synaptic potentials is necessary to excite an action potential and so if spontaneous synaptic potentials occurred of comparable amplitude to the evoked synaptic potential these might well be a significant factor in determining the firing pattern of cells in these ganglia. Large spontaneous synaptic potentials have been observed in many mammalian ganglia receiving multiple inputs, for example, guinea pig sympathetic chain¹⁰, guinea pig superior cervical ganglion⁹ and guinea pig inferior mesenteric ganglion (J. C. Bornstein, unpublished). Thus the output of a ganglion cell may depend not only on activity in the preganglionic nerves but also on the frequency of the spontaneous synaptic potentials released by these nerves. This seems to depend on the past activity of the nerve terminal.

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Effects of β adrenergic blockade on plasma catecholamines in exercise

It is well established that physical exercise causes an increase in sympathetic activity, as shown by rises in the levels of plasma and urine catecholamines¹. This increase in endogenous adrenergic activity can be used to study the effects of sympathetic stimulation. Such studies usually compare a control situation with one in which either α or β adrenergic receptor activity is blocked pharmacologically, thereby allowing study of both the blocked and unblocked receptors. Beta blocking drugs have been used in this way to study changes in carbohydrate metabolism², free fatty acid metabolism³, and blood coagulation and fibrinolysis⁴ induced by exercise.

It has previously been assumed that because adrenergic blocking drugs act by blockade of the receptors, they do not affect the release of the catecholamines, and thus the magnitude of the sympathetic responses. A recent study⁵ has shown, however, that animals subjected to haemorrhagic shock under medication with α and β adrenergic blocking drugs in combination had lower levels of plasma catecholamines than the control animals in spite of an equivalent degree of blood loss and hypotension. Demonstration that single adrenergic receptor blockade also altered the extent of the sympathetic response in a stress situation would be of considerable importance, for in studies involving the use of

TABLE 1 Catecholamine concentrations

		Total catecholamine ($\mu\text{g l}^{-1}$)	Noradrenaline ($\mu\text{g l}^{-1}$)	Adrenaline ($\mu\text{g l}^{-1}$)
		Mean $\pm$ s.e.)	Mean $\pm$ s.e.)	Mean $\pm$ s.e.)
Non medicated	Pre-exercise	0.66(± 0.13)	0.52(± 0.06)	0.14(± 0.16)
	Post exercise	1.80(± 0.32)	0.57(± 0.13)	0.23(± 0.26)
Propranolol	Pre-exercise	0.68(± 0.17)	0.52(± 0.08)	0.16(± 0.13)
	Post exercise	1.41(± 0.37)	0.78(± 0.21)	0.64(± 0.35)
Oxprenolol	Pre-exercise	0.50(± 0.09)	0.29(± 0.14)	0.21(± 0.13)
	Post exercise	1.69(± 0.55)	0.65(± 0.75)	1.04(± 0.55)
LB 46	Pre-exercise	0.78(± 0.20)	0.51(± 0.09)	0.27(± 0.16)
	Post exercise	1.69(± 1.11)	0.84(± 0.38)	0.85(± 0.73)

adrenergic receptor blocking drugs the unblocked receptor could be subjected to different degrees of adrenergic stimulation in the control and test situations. This would invalidate the current assumption that the extent of the sympathetic response is the same in both situations. This study reports the effect of three β adrenergic blocking drugs on the extent of the sympathetic response induced by exercise.

Five healthy males aged between 28 and 37 were studied at weekly intervals for four weeks. On each occasion the subjects had a light breakfast at 0800 h, and then reported to the laboratory at 1000 h. After resting on a couch for 15 min each was seated in a chair to allow removal of blood by venepuncture from an antecubital vein.

Each subject then exercised at 1,050 kilopond M min⁻¹ for 5 min. on a bicycle ergometer. On the first occasion the subjects exercised following the oral administration 2 h previously of 120 mg of oxprenolol (Trasicor, Ciba). In the second week, the experiment was repeated without medication and in the remaining two weeks the subjects were premedicated with 120 mg propranolol (Inderal ICI) and 12.5 mg Pindolol (LB46, Sandoz) respectively.

Immediately following exercise, blood was again taken from an antecubital vein with the subject in a sitting position. Blood samples were immediately put into 10 ml lithium heparin tubes, centrifuged and the red cells separated. The resulting plasma was frozen at -70°C and subsequently analysed by the trihydroxyindole fluorometric method for plasma catecholamine concentrations⁶. Measurements were made of total catecholamines and noradrenaline, the adrenaline level being deduced by difference. The plasma was also analysed for concentrations of the β blocking drugs to confirm adequate β blockade.

Comparison of the resting pre-exercise catecholamine concentrations in the medicated and non-medicated groups showed no difference in the noradrenaline levels. Adrenaline levels in the medicated groups were, however, marginally higher than those in the non-medicated group, but the dif-

ference was not statistically significant. Exercise in both groups caused rises in both plasma adrenaline and noradrenaline levels (Table 1). The extent of the response was however different in the β blocked and control groups. For example, whereas in the non-medicated controls noradrenaline

and by measurement of the plasma levels of all three β blocking drugs.

Most of the volunteers found it more difficult to exercise when medicated with β blocking drugs, but in only one case was there any profound circulatory change. This was observed in one individual medicated with pindolol, who at the end of the period of exercise showed marked bradycardia, cutaneous vasoconstriction and syncope. It is interesting that his rises in adrenaline and noradrenaline (from 0.36 to 1.95 $\mu\text{g l}^{-1}$ and from 0.63 to 1.47 $\mu\text{g l}^{-1}$) were the greatest recorded in this study.

In all forms of exercise the sympathetic nervous system is activated to a greater or lesser degree. The stimuli for this sympathetic response include baroreceptor activation, pain and psychological factors. These results in the release of adrenaline and noradrenaline from the adrenal medulla and nerve endings respectively.

Von Euler⁷ considers that the increase in plasma catecholamines probably reflects compensatory adrenergic activity in non muscular vascular beds, designed to counteract the effects of the vasodilatation induced by exercise in the muscle beds. Activation of the vasomotor centre in exercise is *via* blood pressure homeostatic reflexes⁸. In addition, however, emotional stress occasioned by hard physical exercise could also contribute to the extent of the adrenergic response.

Our studies have demonstrated for the first time a significant difference in the extent of the adrenergic response to exercise in subjects under β adrenergic blockade. The exact reason for the excess adrenergic activity is not clear. The reliability of the fluorometric assay used is now established⁹ and the pre-exercise values in all four groups show that the β blocking drugs themselves have not interfered with the accuracy of the assay. This same observation also confirms that the results cannot be attributed to intrinsic adrenergic activity of the β blocking drugs. Account must be taken of the possible contribution of psychological stimuli to the increased adrenergic response under β blockade, for there is no doubt that in most cases, the volunteers found it more difficult to carry out the workload when medicated with β blockers. Anxiety in the pre-exercise period can, however, be excluded by examination of the resting catecholamine levels.

The most likely cause for the increased adrenergic response in the β -blocked individuals is an exaggeration of the compensatory response described by von Euler⁷. Beta blockade reduces the compensatory increase in cardiac output that normally occurs in exercise by 17% (ref. 10) and this, in association with dilatation in the muscle beds, can lead to a fall in arterial blood pressure¹¹ which activates an increased sympathetic response.

Irrespective of the cause of this increased adrenergic response to exercise under β blockade, the observation is of considerable significance for two reasons. First, it demonstrates that in experiments using exercise to stimulate sympathetic nervous activity to investigate its circulatory or metabolic effects, β adrenergic blockade alters the degree of the adrenergic response, and consequently the stimulation of the unblocked α receptor. Second, the information is of significance in the management of patients on treatment with β adrenergic blocking drugs. These drugs are widely used in the treatment of angina pectoris and hypertension. Our findings indicate that patients being so treated should be warned against undertaking sudden vigorous exercise, as this might result in an excessive adrenergic response which could have deleterious effects.

Beta adrenergic blockade with propranolol, oxyprenolol, or pindolol significantly increases the adrenergic response to exercise as judged by measurement of plasma catecholamine concentrations.

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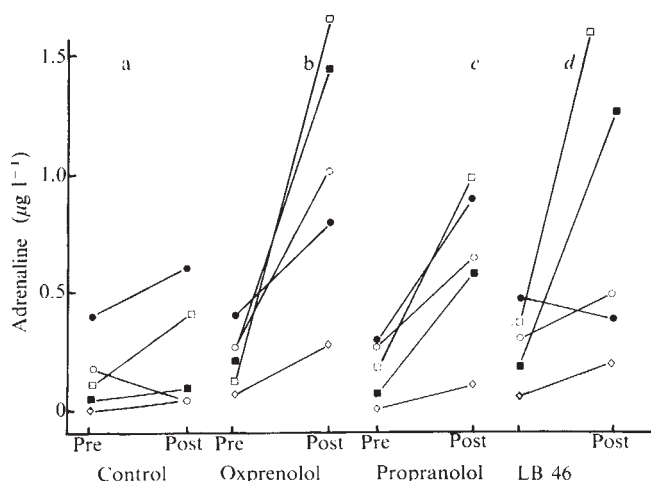


FIG. 1 Effects of adrenergic blockade on plasma adrenaline response to exercise. *a*, Control; *b*, oxyprenolol; *c*, propranolol; *d*, LB 46.

rose from a mean of 0.52 (± 0.06) to 0.57 (± 0.13) $\mu\text{g l}^{-1}$, and adrenaline rose from a mean of 0.14 (± 0.16) to 0.23 (± 0.26) $\mu\text{g l}^{-1}$, the same workload in the group medicated with propranolol produced a rise of noradrenaline from 0.52 (± 0.08) to 0.78 (± 0.21) $\mu\text{g l}^{-1}$ and a rise in adrenaline from 0.16 (± 0.13) to 0.64 (± 0.35) $\mu\text{g l}^{-1}$. Subjects medicated with oxyprenolol and pindolol showed similar results (Table 1, Fig. 1). The difference in the adrenaline response in the β blocked groups and the non-medicated control group was statistically significant ($P > 0.05$). Although the levels of noradrenaline exhibited a similar trend (Fig. 2), the values obtained in the medicated groups were not significantly different from those in the non-medicated group.

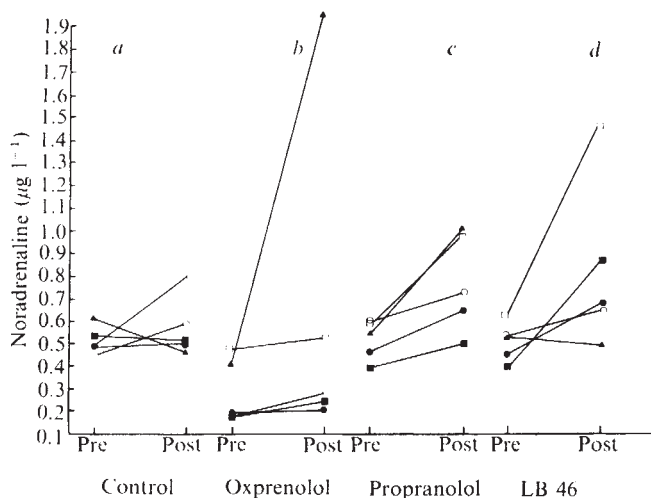


FIG. 2 Effects of adrenergic blockade on plasma noradrenaline response to exercise. *a*, Control; *b*, oxyprenolol; *c*, propranolol; *d*, LB 46.

Evidence of adequate β blockade in the medicated groups was demonstrated both by a reduction in the degree of exercise-induced tachycardia evident in the nonmedicated group

concentrations of oxprenolol, and Sandoz Ltd for arranging statistical advice.

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Glutamate antagonists at a crayfish neuromuscular junction

As Kravitz *et al.*¹ have pointed out, glutamic acid is at present the only candidate for the excitatory transmitter at crustacean neuromuscular junctions. The evidence is that^{1,2} glutamic acid is the most potent excitatory substance present in the crustacean central nervous system (CNS), whole peripheral nerves and isolated excitatory axons^{1,3-5}; that the glutamic acid content in nerve extracts accounts for the whole of their excitatory activity on crustacean muscles¹, and that the receptors to glutamate are localised at the same junctional spots as the receptors to the natural excitatory transmitter⁶. Also, both glutamic acid and the natural transmitter produce similar changes in the permeability of the postjunctional muscle membrane⁷⁻⁹ and in spite of a large background leakage, a significant release of glutamic acid can be evoked by stimulation of excitatory nerves¹. There is also a selective uptake of glutamic acid at the neuromuscular junction which could provide an inactivation mechanism¹⁰. There is, however, no information on whether glutamate receptors have the same pharmacological properties as the receptors of the natural excitatory transmitter.

The main object of the work reported here has been to screen a series of compounds for a specific antagonist to glutamic acid. We have found some drugs which specifically block both the responses to iontophoretically applied glutamate and the excitatory junctional potentials (EJPs) recorded from the same crayfish muscle fibres.

We used the slow flexor abdominal muscle fibres¹¹ from the crayfish *Procambarus clarkii*. These muscles are located in the anterior wall of each abdominal segment, each side of the midline, just beneath the integument. One insertion is on a sternite and the other on an integumental ridge. The superficial layers of the anterior wall of an abdominal segment were removed together with both sternites limiting the segment and placed in a perspex chamber through which van Harreveld's solution was continuously circulated. The

integument covering the muscle was first excised to allow the transillumination of the muscles and the two sternites were then fixed to the bottom of the chamber, without stretching the muscle fibres, in order to expose the posterior face of the slow flexor muscles. Single fibres were impaled with two independent KCl-filled micropipettes separated by 100-200 μ m. One pipette was used for recording through an amplifier of unit gain connected to a 280 Brush pen recorder; the other was used to pass constant current pulses across the membrane for measuring the input resistance of the fibre and/or steady current for setting the membrane potentials to desired levels. Glutamate ions were applied iontophoretically on to the muscle fibres as an anion from a third micropipette filled with a 2 M monosodium glutamate solution at pH 8.0. When necessary, GABA was also applied iontophoretically as a cation, from another pipette filled with a 1 M GABA solution at pH 3.5.

To study the effect of the drugs on the EJPs advantage was taken of the fact that the slow flexor abdominal muscles fibres receive synaptic inputs from several 'spontaneously' firing motoneurons¹². A whole abdomen preparation was therefore set up in the chamber, the integument of one of the segments was removed and the fibres were impaled with a micropipette. In this preparation, the connections of the nerve cord with the muscles remained intact, and a barrage of 'spontaneous' EJPs could be recorded (Fig. 1). In all the experiments, the drugs tested were dissolved in the van Harreveld solution and perfused to the preparation.

When glutamate is applied to a slow flexor muscle fibre three main sensitive regions, separated by insensitive areas, are revealed: one at each end of the fibres near the insertions and a large field in the central parts of the fibre. The responses to glutamate consist of fast depolarisations (Fig. 2a),

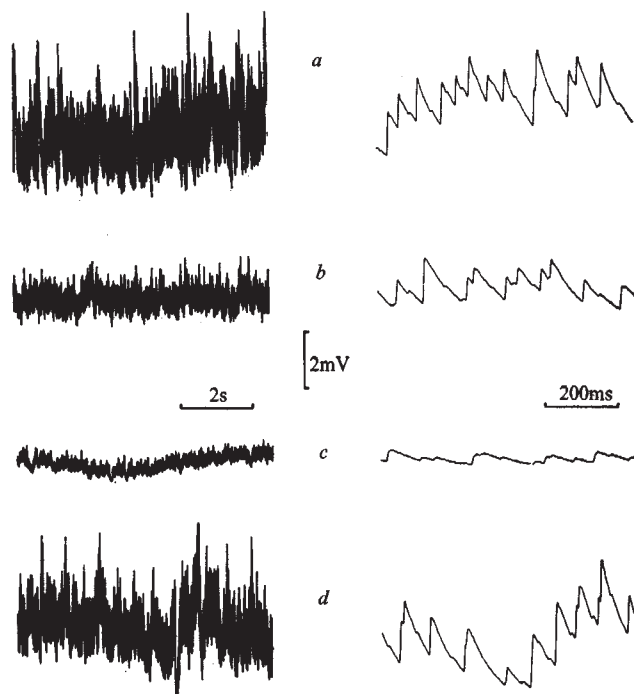


FIG. 1 Isolated crayfish abdomen preparation. The EJPs recorded from an abdominal slow flexor muscle fibre result from the 'spontaneous' firing of several motoneurons. In each horizontal row, the same synaptic activity was recorded at two different speeds. *a*, EJPs discharge when the preparation is bathed in a normal medium. *b*, After perfusing to the preparation a 10^{-3} M solution of L-glutamic acid γ -methyl ester, the amplitude of the EJPs is decreased. *c*, Decrease in the amplitude of the EJPs is almost complete after the concentration of the antagonist to 10^{-2} M. Notice in *b* and *c* that there is no evident alteration of the frequency of discharge of the EJPs. *d*, Recovery after prolonged washing.

easily desensitised by repeating the iontophoretic application, and showing the same time course and properties as the ones described by Takeuchi and Takeuchi⁶ in the dactyl muscles.

Of 56 drugs (glutamic acid structural analogues, enzyme inhibitors, drugs showing effects on other synapses), eight substances were found to block the glutamate responses (Table 1). The mechanism of their blocking action was analysed and their relative potency evaluated. The γ -methyl ester of L-glutamic acid (L-Glu- γ -ME) was the most potent of the drugs tested, followed by other methyl and ethyl esters of L-glutamic acid, piperazine, strychnine and phenylurea. The effect of these compounds on the input resistance of the muscle fibres was tested and only phenylurea and coumarin were found to increase consistently the resting membrane conductance. This effect was probably independent of the blocking effect since after these substances were

TABLE 1 Eight substances which block the glutamate response

Compound	Relative blocking efficacy	Effect on membrane conductance	Effect on GABA receptors
L-glutamic acid γ -methyl ester	100	No	No
L-glutamic acid dimethyl ester	90	No	No
L-glutamic acid monoethyl ester	60	No	No
L-glutamic acid diethyl ester	50	No	No
Piperazine	90	No	Blocks
Strychnine	80	No	No
Phenylurea	80	Increases	No
Coumarin	60	Increases	No

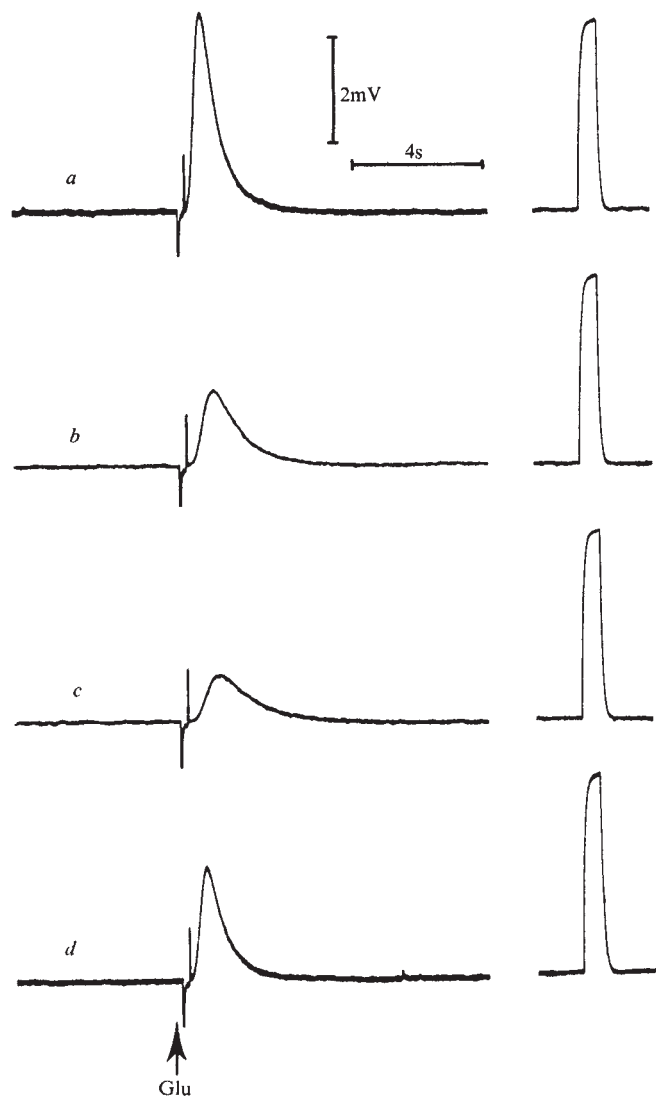


FIG. 2 A series of iontophoretic applications of glutamate on to an abdominal slow flexor muscle fibre of *Procambarus*. The vertical bars mark the duration of the application. At the right of the recordings, a series of electrotonic potentials obtained after each glutamate response by passing current pulses across the fibre membrane. *a*, Glutamate response and the electrotonic potential in a normal medium. *b*, Glutamate response decreases in amplitude after perfusion to the preparation of a 10^{-3} M solution of L-glutamic acid γ -methyl ester. *c*, Response to glutamate is almost blocked under action of a 2×10^{-3} M solution of the antagonist. In both *b* and *c* there is no change in the amplitude of the electrotonic potentials, that is, of the membrane resistance. *d*, Onset of recovery of the glutamate response after removal of the blocking agent.

removed the membrane input resistance rapidly recovered its control values, whereas the recovery of the glutamate responses was much slower. Because of these non-specific effects on the membrane conductance these two substances were unsuitable for analysing the synaptic inputs.

In addition, piperazine, which did not affect the muscle membrane conductance, blocked the responses not only to glutamate, but also to GABA. We considered that this made piperazine not worth further investigation. Finally, strychnine was found to be an effective antagonist of glutamate on these muscle fibres at concentrations of 10^{-3} to 10^{-2} M, without showing effects on the membrane conductance or the GABA responses. But since strychnine has been shown to block other receptors in other preparations (glycine receptors in vertebrate spinal neurones¹³ and acetylcholine receptors in molluscan neurones¹⁴) and to interfere with the release of transmitter in vertebrates¹⁵ and invertebrates¹⁶, we did not use it in the experiments on the EJPs.

The most suitable drugs for such analysis were therefore the esters of L-glutamic acid, in particular L-Glu- γ -ME. Figure 2(*b* and *c*) shows that at concentrations up to 2×10^{-3} M it blocks almost completely the response to glutamate without affecting the membrane conductance of the muscle fibre. Even at concentrations of 10^{-2} M no effect on the input resistance was observed. The recovery shown in Fig. 2*d* is only partial but after prolonged washing the effect was totally reversible. On the other hand, the same concentrations of antagonist did not affect the responses of the fibres to the iontophoretic application of GABA (Fig. 3*b*).

Haldeman and McLennan¹⁷ working with spinal and cuneate neurones found that the diethyl ester of L-glutamic acid was the most potent blocker of the series, whereas the methyl esters had agonistic effects. This reversed pattern suggests that the receptors in the vertebrate CNS and on crustacean neuromuscular junctions could be different.

The effects of L-Glu- γ -ME were assayed in the 'spontaneous' EJPs recorded in the slow flexor muscle fibres in an *in vitro* abdomen preparation (see above). In Fig. 1*a* a barrage of EJPs was recorded in these fibres at two different speeds. When L-Glu- γ -ME was applied to the preparation at concentrations of 10^{-3} M (Fig. 1*b*) and 10^{-2} M (Fig. 1*c*) the amplitude of the EJPs gradually decreased without evident alteration of their frequency. This effect was reversible (Fig. 1*d*) after prolonged washing.

These experiments show that L-Glu- γ -ME blocked both the responses to the iontophoretic application of glutamate and the EJPs recorded from the same muscle fibres, without altering the input resistance of the fibres, the frequency of the neuronal firing or the conduction velocity of the motor axons. Nevertheless, the possibility that this compound acts on the quantal content of the EJPs must be investigated in further experiments.

We conclude that in spite of its low affinity for the glutamate receptors, L-Glu- γ -ME is a highly specific antagonist of glutamate on the crayfish neuromuscular postjunctional mem-

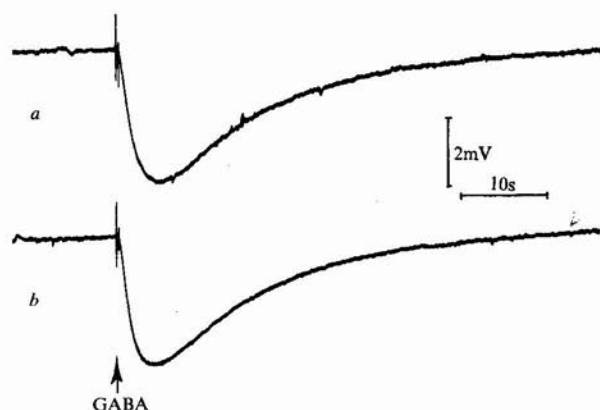


FIG. 3 L-glutamic acid γ -methylester does not affect the GABA responses of an abdominal slow flexor muscle fibre. *a*, Control hyperpolarising response to the iontophoretic application of GABA. *b*, GABA response remains unchanged after perfusing to the preparation a 5×10^{-3} M solution of the glutamate antagonist.

brane. The observation that it also blocks, very probably at the postsynaptic level, the generation of the EJPs is a new argument in favour of a transmitter role of glutamate in these neuromuscular junctions.

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Trichodermin resistance—mutation affecting eukaryotic ribosomes

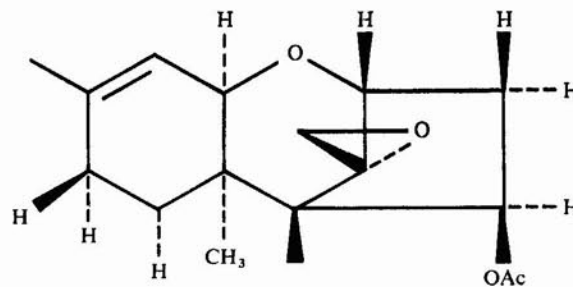
GENETIC studies of ribosomes in prokaryotes such as *Escherichia coli* have been facilitated by the use of mutants resistant to antibiotics; mutations which affect either subunit of the ribosome are known¹. In eukaryotes, genetic informa-

TABLE 1 Cross resistance between trichothecenes

Drug	CLP-1 (resistant) 100% = 13.2	A224A (sensitive) 100% = 63.1
Control		
Trichodermin (20 $\mu\text{g ml}^{-1}$)	100%	95%
T-2 Toxin (5 $\mu\text{g ml}^{-1}$)	67	26
T-2 Toxin (1 $\mu\text{g ml}^{-1}$)	82	53
Verrucaric A (5 $\mu\text{g ml}^{-1}$)	62	18
Verrucaric A (1 $\mu\text{g ml}^{-1}$)	69	27
Nivalenol (5 $\mu\text{g ml}^{-1}$)	99	40
Nivalenol (1 $\mu\text{g ml}^{-1}$)	95	55

S. cerevisiae strains A224A and CLP-1 were grown on a medium containing yeast extract-peptone-dextrose buffered to pH 5.8 with sodium succinate to about 3×10^7 cells ml^{-1} . The cells were collected, washed with ice-cold distilled water, resuspended in an equal volume of 100 mM Tris-50 mM KCl-5 mM Mg acetate-1 mM DTT (TKMD), and passed two times through a French pressure cell at 16,000–20,000 pound inch^{-2} . The suspension was centrifuged twice for 10 minutes at 18,000 r.p.m. in an SE-12 rotor (34 Kg). The top two-thirds of the supernatant was centrifuged for 2 h at 42,000 r.p.m. in a Ti50 rotor. The supernatant was removed avoiding the fluffy layer which was poured off and the pellet was resuspended in TKMD and 4 M NH_4Cl was added to a final concentration of 0.3 M and stirred for 30 min. The ribosomes were pelleted at 42,000 r.p.m. for 2 h, resuspended in TKMD and dialysed overnight against TKMD containing 0.6 mM β -mercaptoethanol. A supernatant fraction was prepared by precipitating the postribosomal supernatant with 70% ammonium sulphate and collecting the precipitate at 34,000 g for 20 min. The pellet was resuspended in one-third the original volume of TKMD and dialysed overnight in TKMD containing β -mercaptoethanol. Phenylalanine incorporation was performed in mixtures containing 50 mM Tris-HCl, pH 7.6, 100 mM KCl, 18 mM Mg acetate, 500 $\mu\text{g ml}^{-1}$ tRNA, 3.3 mg ml^{-1} creatine phosphate, 260 $\mu\text{g ml}^{-1}$ creatine kinase, 2 μM ^{14}C -phenylalanine (10 $\mu\text{Ci ml}^{-1}$), 1.0 mM ATP, 0.1 mM GTP and 500 $\mu\text{g ml}^{-1}$ poly (U). Incubations were carried out at 30 °C for 30 min and terminated with 1 ml of 10% TCA. The reaction mixtures were filtered through GF/C glass fibre filters and washed five times with 3 ml of 5% TCA. Filters were dried and counted in a toluene-based scintillation fluor. Figures are expressed as percentage of control activity which is given in picomol of ^{14}C -phenylalanine incorporated.

tion on the translation apparatus has come mainly from studies of temperature-sensitive mutants^{2,10}, and also cycloheximide resistance, which is known to be a property of the 60S subunit³. Recent studies with resistance to the alkaloid cryptoleurine have shown that *Saccharomyces cerevisiae* strains resistant to this compound have altered ribosomes⁴.



Trichodermin

Trichodermin is a fungal toxin which belongs to the general class of 12, 13-epoxytrichothecenes; these compounds inhibit protein synthesis in eukaryotes but not prokaryotes (for a review, see ref. 5). The mode of action of these compounds on protein synthesis *in vivo* has been studied and the trichothecenes can be divided into two classes on this basis⁶. Some of the trichothecenes (such as verrucaric A and nivalenol) inhibit a step related to or shortly after initiation, while trichodermin affects elongation and/or termination by interfering with the peptidyl transferase reaction on the ribosomes. We now report the isolation of a spontaneous mutant of *S*

TABLE 2 Localisation of resistance to nivalenol on *S. cerevisiae* ribosomes

(a)	Ribosomes	Supernatant	Control	+ Nivalenol (5 µg ml ⁻¹)	% Activity
	A224A	A224A	82.4	19.0	23
	A224A	CLP-1	51.5	17.0	33
	CLP-1	A224A	33.5	32.8	98
	CLP-1	CLP-1	8.9	8.7	98
(b)	Subunit source				
	40S	60S			
	A224A	A224A	69.8	22.3	32
	CLP-1	CLP-1	33.7	34.4	102
	CLP-1	A224A	84.2	23.6	28
	A224A	CLP-1	31.2	31.2	100
	A224A	—	—	—	—
	—	A224A	4.2	—	—
	CLP-1	—	3.9	—	—
	—	CLP-1	6.0	—	—

Ribosomal and supernatant fractions were prepared as described in the legend to Table 1. Free ribosomes were prepared as described by van der Zeijst *et al.*⁹ and subunits were separated by centrifugation through a 10–30% sucrose gradient in 50 mM Tris-HCl, pH 7.6 containing 500 mM KCl and 5 mM Mg acetate for 7 h at 22,500 r.p.m. in a SW-27 rotor at 15° C. The supernatant fraction used with the subunits was derived from the wild-type sensitive strain A224A. 0.13–0.20 A 260 units of each subunit was used in reaction. The incorporation of phenylalanine was assayed as in Table 1. Activity is expressed as total picomol of ¹⁴C-phenylalanine incorporated. An independently isolated mutant, CLP-8, gave similar results.

cerevisiae A224A which is resistant to 20 µg ml⁻¹ of trichodermin and show that this mutant has an altered 60S subunit.

Ribosomes and supernatant fractions from a strain sensitive to trichodermin, A224A, and a strain resistant to trichodermin CLP-1, were prepared and tested for their response to trichodermin and related trichothecenes in Poly(U)-directed polyphenylalanine synthesis. Ribosomes from the sensitive strain were strongly inhibited by several of the trichothecenes (Table 1). In agreement with previous reports⁷, trichodermin was not an effective inhibitor of the poly(U) system. Polypeptide synthesis in extracts of the trichodermin-resistant strain was markedly resistant to nivalenol, and since trichodermin is not an effective inhibitor in the poly (U) system, we have used nivalenol to determine which component of the protein-synthesising system is altered in the strain resistant to trichodermin.

Table 2 shows that resistance to nivalenol in *S. cerevisiae* CLP-1 was associated with the ribosomes; specifically with the 60S subunit. As a further demonstration that trichodermin resistance was associated with the ribosomal fraction of strain CLP-1, we tested the effect of trichodermin on

TABLE 3 ³H-Puromycin-induced release of polypeptide chains from yeast polyribosomes

	– Trichodermin	+ Trichodermin	% Inhibition
A224A			
Polysomes	2.2, 2.5	0.7, 0.6	67.3
CLP-1			
Polysomes	3.2, 1.1	2.3, 0.7	30.9

Yeast spheroplasts were prepared as described by Hartwell and McLaughlin¹⁰, substituting 1 M sorbitol for the 0.4 M MgSO₄. They were collected by centrifugation, resuspended in TKM and lysed with 0.5% sodium deoxycholate and 1% Brij 58 (Atlas Chem. Co.). The lysate was layered on a discontinuous gradient of 20% sucrose–60% sucrose in TKM, and centrifuged at 35,000 r.p.m. for 4 h in the Ti50 rotor. The pellet was resuspended in TKM. Assays were performed as described by Pestka *et al.*¹¹ except that GF/C glass fibre filters were used to collect the insoluble peptidyl-puromycin fragments. Trichodermin was present as indicated at 20 µg ml⁻¹. Reactions were incubated at 30° C for 5 min. Activity is expressed as total picomol of ³H-puromycin incorporated into peptidyl-puromycin.

puromycin-induced release of nascent peptide chains on polyribosomes (puromycin reaction). In the sensitive strain the puromycin reaction was inhibited in the presence of trichodermin, whereas the puromycin reaction on polyribosomes of *S. cerevisiae* CLP-1 was substantially resistant to trichodermin (Table 3).

As well as providing important genetic information on the structural components of ribosomes, antibiotic-resistant mutants also provide clues to the mode of action of an inhibitor. The demonstration of trichodermin resistance as a property of the 60S subunit of resistant strains is consistent with the notion that trichodermin inhibits the peptidyl transferase reaction (a property of the 60S subunit⁸). It has been suggested previously that it could act by preventing access of the aminoacyl-tRNA acceptor to the peptidyl transferase centre⁷.

Genetic studies have shown *S. cerevisiae* to have at least 17 chromosomes. Cycloheximide resistance, *cyh2*, a property of the 60S subunit, maps on chromosome VII² and cryptopleurine resistance, *cry1* (another ribosomal alteration) maps on chromosome III (ref. 4 and P. Grant, unpublished observations). Genetic analysis of CLP-1 indicates that trichodermin resistance is not linked to *cyh2* or *cry1*. Although there is no evidence that these mutations affect ribosomal proteins and not rRNA or modifying enzymes, the organisation of structural genes for ribosomes in simple eukaryotes such as *S. cerevisiae* may prove to be different from the ribosome gene 'cluster' found in prokaryotes such as *E. coli*. Recently, the genes for ribosomal proteins of *Drosophila* have been mapped and all ribosomal protein genes may be located within a 'cluster' in this eukaryote¹². In addition, the *Drosophila* ribosomal protein genes are linked to rRNA genes. In *S. cerevisiae* it has been shown that 70% of the rRNA is coded for by chromosome 1 (ref. 13). Trichodermin resistance is not linked to *adel* on this chromosome; therefore none of the three mutations, cycloheximide resistance, cryptopleurine resistance, or trichodermin resistance, which are known to affect ribosomes in *S. cerevisiae* are linked to the known rRNA genes or to each other.

This investigation was supported by a grant from the National Institutes of Health. Drs W. O. Godtfredsen, F. Strong and C. Tamm provided gifts of fungal toxins.

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book reviews

Animal interactions

Models in Ecology. By J. Maynard Smith. Pp. xii+146. (Cambridge: London, January 1974.) £3.70; \$10.50.

ECOLOGY suffers from a surfeit of fascinating, but apparently unrelated observations, superimposed upon an acute shortage of general theories. An inevitable consequence has been the growing importance of general, precise, rather simple models which may provide insights into the workings of the real world, without its baffling complexity. This book provides a particularly good example of the approach, although it is by no means a general account of the ways in which models have been, and are being used in ecology. The problems which it chooses to examine range from predator-prey systems (chapters 2, 4, 6 and part of 11), the importance of time delays in population dynamics (chapter 3), competition (chapters 5 and 9), stability and complexity (chapters 7, 8, 10 and 11) and territorial behaviour (12), with the underlying theme throughout being the problem of stability in ecological systems. Virtually all the models are in the form of deterministic differential equations, the justification for using deterministic models being discussed briefly in chapter 1. In a comparatively small number of instances difference equations are used, while Leslie matrices are mentioned so briefly in chapter 3 that their inclusion hardly seems worthwhile.

As Maynard Smith points out, "the purpose of mathematics is to render the assumptions lying behind the arguments more explicit". But that is only the first step, albeit an important one. Conclusions derived through use of mathematics, and hence the assumptions themselves, should obviously be tested repeatedly against the real world, and even more importantly the models should suggest new insights and suggest new observations that are worth making. There are a disappointingly few places in this book where really new insights emerge, although it provides additional information and very useful summaries of a number of previously fairly well worked ideas. Material that is substantially new is to be found in chapter 6, which considers the effect of migration between sub-populations on the stability of predator-prey systems. It is a great pity that the logic used in setting up the model (pages 75-83) is not more fully explained. It seems to contain some cu-

rious assumptions, including the one that the addition of predators will make the prey in a subpopulation reach a larger population size faster than would be the case in the absence of predators. This does not seem reasonable. It is also a pity that no attempt has been made to find an analytical solution to the migration models, at least for the case of the simple model.

Chapter 10 includes a new analysis of a simple food web which attempts to provide a partial answer to the important question of how the number, and strengths, of the connections may influence the stability of the component populations. Again, an analytical solution is not attempted (and in this case one may not exist), but a simulation provides some interesting conclusions. Finally, chapter 12 provides a particularly good example of how an extremely simple model (in this case concerned with the influence of territorial behaviour on population density in birds) can provide some very interesting new insights into a well studied, but little understood, phenomenon.

In a number of other places in the book, the analysis may not be particularly new, but what it does do is explain well the main features of, or drawbacks and advantages in the types of analysis used by other workers. For example, it has some interesting comments to make about the 'standard' competition equations, and it provides an excellent summary of the major drawbacks inherent in the application of statistical mechanics to community modelling. Despite the sophistication of the mathematics, efforts in this direction would seem to be totally misdirected.

One of the main problems with the book is the patchiness with which it makes successful contact with the real world. Indeed this is partially acknowledged by the author, and by itself is not a major criticism, unless the reader (in making the contact for himself) finds a number of the models inadequate. Thus, there are rather sweeping generalisations being made about predator-prey systems in general, that are based almost entirely on models of predators which feed on only one species of prey, without questioning whether this is actually typical of most real predators. The fact that it isn't is never mentioned.

Nor are some of the actual attempts to relate observations and models equally successful. Some, like the analysis of Nicholson's blowflies in chapter 3 seem

to be good, but others, like the attempts to relate the stability properties of community models to Elton's and Watt's observations seem to lose sight of the obvious point that Elton and Watt are largely talking about population fluctuations, and the models are dealing with neighbourhood stability. These are not necessarily the same thing. Until ecologists build community models which consider global stability, and until they have experimental data that show whether the fluctuations one observes in nature are really unstable, or whether they merely represent, say stable limit cycles, movements towards equilibria with long damping times and continual disturbance, or what have you, most of the elegant discussion on pages 112-5 is not relevant.

It is certainly not a book for someone without a fairly good grounding in ecology. Equally it is not particularly suitable for someone who wants to learn how to apply certain techniques. One could not, for example, find out how to go about solving the general neighbourhood stability properties of a differential equation model. What it does do is show how apparently complex ecological problems can often be approached by rather simple models, and how such models can be used to 'get the feel' of the problem. If it encourages more practicing ecologists to do this it will have served a useful purpose.

J. H. LAWTON

Mathematical magnetism

An Introduction to Electromagnetic Theory. By P. C. Clemmow. Pp. xi+297. (Cambridge University: London, 1973.) \$10.

IN explaining his reasons for adding another text to the many which already deal with electromagnetism at undergraduate level, Dr Clemmow makes the point that competition from other topics in a widening curriculum leaves less time than formerly for a student to master the fundamentals of electromagnetism and this calls for a more crisp approach than has been usual. This idea determines both his choice of material and the manner of presentation. The material is pared down to theoretical essentials, very little being allowed in purely to illustrate or for reinforcement, while the style is disciplined and economical, making each point once only, and that in a rather austere mathematical way. Within this framework,

the route through the theory is not unconventional. Vector algebra and MKS units are used throughout and Maxwell's equations introduced fairly soon, to be followed by chapters dealing with electrostatics, magnetostatics, waves and properties of media. Each chapter is followed by a set of problems, mostly of the 'show that' variety—otherwise no answers are provided.

It is consistent with the author's aim that there are few concessions to any weakness in the reader. It is assumed that he is fully conversant with vector algebra and needs little assistance in visualising the significance of the equations. Magnetic field lines for example are mentioned first in Chapter 4 and so are not employed in the fairly lengthy discussion leading to Maxwell's equations in chapter 2. The approach of this discussion is again mathematical as opposed to physical. For instance, the equation $\text{div } B = 0$ appears as a consequence of $B = \text{curl } A$ rather than as a statement of the absence in nature of magnetic poles.

It is difficult to judge how big is the gap which the book is designed to fill. Well prepared students will appreciate the lack of distractions and the numerous mathematical points succinctly made. It would hardly be appropriate though for those, perhaps more typical students, who find they need to learn

mathematical methods and physical theory together and also need to surface frequently from abstract depths to reinforce new ideas with physical pictures and test them in the context of familiar practical applications.

S. CLOUGH

Raman spectra

The Raman Effect. Vol. 2: Applications. Edited by A. Anderson. Pp. xi+405-1033. (Dekker: New York, July 1973.) \$45.

THE initial impact of this book may well be governed by its price since \$45 is a particularly excessive sum to pay for a textbook. But on closer acquaintance it is seen to be rather less the extortion of funds from university libraries than it might appear since the book consists of two or three sub-books in the form of reviews, 100-200 pages in length, which are both authoritative and comprehensive.

The first chapter by Tobias gives applications of Raman spectroscopy to inorganic chemistry, describing experimental techniques and how to interpret results to obtain structural information. There are also sections on solution equilibria and fused salts and it provides a most valuable review. It is followed by a chapter on electronic Raman transitions from Koningstein and Mortensen. This is a very concise account, which contains the essentials of the use of the technique in identifying low-lying electronic states in crystals containing rare-earth ions. The next chapter is a treatise by Weber on high resolution Raman spectra of gases. This very complete account discusses the theory of rotational Raman, and its use in molecular structure determination. It also gives useful information on experimental techniques in detecting the weak signals characteristic of gas phase work.

Finally, there are two chapters on the spectra of crystals. A short introduction to the study of molecular crystals is given by Savoie who outlines the theoretical basis of the treatment of pure solids. This chapter mainly concerns crystals of simple molecules such as HCN and CF_4 . The last chapter by Wilkinson is an outstanding review of the spectra of ionic, covalent and metallic crystals. Theoretical principles are lucidly explained in the early part of the chapter and the author then discusses assignments and experimental data for a large number of crystalline systems classified into different types. In each case the analysis is presented together with a discussion of available data. In addition there is a section on scattering from Landau levels, magnons and states other than phonons.

This collection of very authoritative articles will provide an invaluable refer-

ence text for workers in the field of Raman spectroscopy. The price of the book will ensure that few individuals will purchase it. This is particularly emphasised since this volume does not contain a chapter devoted to the general theory of the Raman effect and thus its companion volume is required. None the less it is a well produced book and it fills several gaps in the literature with authority.

A. J. McCaffery

Master class

Molecular Techniques and Approaches in Developmental Biology. Edited by Maarten J. Chrispeels. Pp. xii+306. (Wiley-Interscience: New York and London, October 1973.) £8.25.

THE mixed bag of articles making up this book originated in the La Jolla Summer Workshops on Molecular Techniques in Developmental Biology. Each chapter is based on a practical project run by an invited scientist, to teach the techniques used in his own research. The net result is a book which lacks coherence and covers only a limited amount of ground but, nevertheless, contains an enormous amount of very practical information, often in the form of step by step instructions with hints on how to adapt the method to other tissues. Obviously such a book can never be a real substitute for learning at the bench of a master and absorbing his or her approach towards developmental problems, but in the absence of opportunities for such personal contact it is certainly the next best thing.

The more prosaic of the eleven chapters deal with the fractionation of sub-cellular organelles, the separation of isozymes by gel electrophoresis and proteins by gel filtration, and the isolation of DNA from eukaryotic cells. Other chapters provide very detailed accounts of how to measure the number of progesterone receptor sites in rat uterus, how to calculate the real rate of RNA synthesis in sea urchin embryos incubated with ^3H -adenosine by measuring the specific radioactivity of the ATP pool, and how to study changes in the population of transfer RNA species in developing tissues. The chapters I found most interesting were those by Pong and Loomis on the isolation of RNA polymerases from *Dictyostelium*, and by Bob Church on the theory and methodology of DNA-DNA and DNA-RNA hybridisation as applied to nucleic acids from cells of higher animals. The latter is a particularly lucid description of some very complex techniques and covers a lot of ground, including the increasingly important method of RNA-DNA hybridisation in a vast excess of DNA.

BRIGID HOGAN

THE HISTORY OF QUANTUM THEORY

Friedrich Hund

Professor at Göttingen University

Translated by Gordon Reece
M.Sc., Imperial College, London

Professor Hund — one of the few remaining scientists with a first-hand knowledge of the development of quantum theory in physics and chemistry between 1900 and 1927 — describes comprehensively and accurately the major discoveries, ideas and the people in this exciting period of scientific history.

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matters arising

Waste and the Pacific

SIR,—The letter which appeared in *Nature* concerning the distribution of tar along latitude 35°N in the Pacific Ocean¹ raises the question of the cause of the pronounced peaks which occur.

One of the explanations advanced is that meanders in the Kuroshio current could be responsible for the distribution. If this were the case, the distribution would have peaks corresponding to the meander wavelengths, which are typically 200 km to 400 km (2° to 4° longitude). This is unlikely to be correct, since the three pronounced peaks are separated by distances of 2,000 km (20° longitude) and 1,300 km (13° longitude).

Another possible explanation of this distribution is that the intense Kuroshio current may generate a series of Rossby waves, which can propagate across the entire Pacific Basin².

In a numerical model recently developed by myself (to be published) a surface layer, 100 m deep and in an idealised basin, was subjected to a zonal

wind stress typical of winter in the Northern hemisphere. The energy dissipation was parameterised to occur

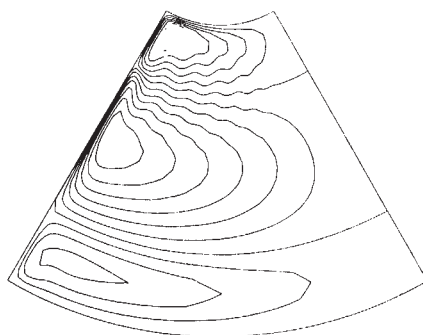


FIG. 1 The pattern of streamlines induced by a steady wind field in a hypothetical ocean basin, which extends from the equator to 60°N, and has a width of 60° longitude. The flow of the water is parallel to the streamlines and its intensity is proportional to the concentration of streamlines. The intense flow on the western edge of the basin is representative of the Kuroshio current in the North Pacific and the Gulf Stream in the North Atlantic.

over the continental shelves. The resulting steady circulation produced (Fig. 1) shows 'ripples' in the eastward flowing current between the sub-arctic gyre and the sub-tropical gyre (30°N to 55°N). The wavelengths in this particular example decrease from 1,300 km (13° longitude) in the western half of the basin to 600 km (6° longitude) in the east. The amplitude of meridional velocity at 35°N decreased from 8 cm s⁻¹ in the west to 4 cm s⁻¹ in the east.

I suggest, therefore, that the observed distribution of tar may be explained more satisfactorily in terms of the type of waves predicted by the model.

Yours faithfully,

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¹ Wong, C. S., Green, D. R., and Cretney, W. J., *Nature*, **247**, 30 (1974).

² Moore, D. W., *Deep Sea Research*, **10**, 735 (1963).

obituary

G. P. Kuiper

GERARD PETER KUIPER died suddenly on December 24, 1973, after a long and greatly distinguished career of ardent devotion to astronomy. He has left his mark by his achievements in observational and theoretical research and in developing new instruments, and in many other ways—as a director and founder of research institutes, as a writer and editor in the grand manner, as from the outset a prominent collaborator in the NASA lunar and planetary programmes which he called “the greatest scientific venture of history.”

Kuiper was born on December 7, 1905 in the Netherlands. In 1933 he gained his doctorate in Leiden for a thesis on binary stars with the renowned Ejnar Hertzsprung as his adviser. He then went to work mainly on the same subject at Yerkes Observatory, where Otto Struve had assembled perhaps the most brilliant

team of young astronomers in existence. After spending the year 1935–6 at Harvard, Kuiper returned to Yerkes as a staff-member and, apart from civilian scientific war services—he had become a United States citizen in 1937—he remained until 1960, twice serving as Director of the Yerkes and McDonald Observatories 1947–49, 1957–60. He then moved to the University of Arizona in Tucson, where he founded and directed the Lunar and Planetary Laboratory and its Catalina Observatory with an armoury of large telescopes for planetary work. These included the well-known 61-inch high resolution telescope specially adapted for observations in the near infrared, one of the many fields in which Kuiper was a pioneer. He relinquished some administrative responsibilities in 1973, but at the time of his death he was planning fresh developments of his scientific work. In the United States and abroad, Kuiper received numerous hon-

ours for his contributions to astronomy; the Royal Astronomical Society elected him an Associate in 1951.

As long ago as 1937, Kuiper's paper on Hertzsprung-Russell diagrams of stellar clusters pointed the way to much subsequent empirical work on stellar evolution. His pioneering work with Stromgren and Struve on models for close binary stars initiated what is still one of the most lively branches of stellar astrophysics. His classical papers of 1938 on the empirical mass-luminosity relation and of 1942 on “the nearest stars” remained for over a decade the main compendia of empirical stellar parameters.

Kuiper's dominating lifelong interest was, however, the solar system. He discovered two new satellites Miranda (Uranus) and Nereid (Neptune), the atmosphere on Saturn's satellite Titan, and the asteroid that bears his name. From 1960 onwards he produced (with

collaborators) four successive atlases of the Moon, based first on observations from Earth and then from space vehicles, and these played a vital role in choosing sites for Apollo landings. All this effort was inspired by the hope of ultimately elucidating the origin of the solar system.

With the astronomer Barbara M. Middlehurst, Kuiper edited the two famous series *The Solar System* (four of its five volumes are published), and *Stars and Stellar Systems* (seven of its nine volumes are published). Indeed, astronomers have seemed to be more excited when a new Middlehurst-Kuiper volume appeared than they were when quasars or pulsars were discovered.

Announcements

Appointments

The Council of the University of Bristol has appointed D. C. Smith, of the Department of Agricultural Science of the University of Oxford, to the Melville Wills Chair of Botany.

Erratum

In the article "Fluctuations in oil flow before and after earthquakes" by E. Arieh and A. M. Merzer (*Nature*, **247**, 534; 1974) the former author is at the Seismological Laboratory, Geological Survey of Israel, Jerusalem and the latter at the Department of Environmental Sciences, University of Tel Aviv.

International Meetings

April 15-19, **9th International Symposium on Remote Sensing of the Environment** (Extension Service, Conference Department, The University of Michigan, Ann Arbor, Michigan 48104)

April 16-17, **The Application of Chemical Engineering to the Treatment of Sewage and Industrial Liquid Effluents** (Dr D. Geldart, Postgraduate School of Studies in Chemical Engineering, University of Bradford, Yorkshire)

April 16-18, **Optical and Acoustical Microelectronics** (Jerome Fox, Polytechnic Institute of Brooklyn, MRI Symposium Committee, 333 Jay Street, Brooklyn, New York 11201)

April 16-18, **University of Dacca Physics Symposium** (Professor A. M. Harnar Rashid, Organising Secretary,

Physics Department, University of Dacca, Dacca 2, Bangladesh)

April 17-19, **2nd Conference on Negative Ions** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX)

April 18-19, **Annual Congress and Scientific Exhibition of the British Institute of Radiology** (The General Secretary, The British Institute of Radiology, 32, Welbeck Street, London W1M 7PG)

April 22-24, **1st International Conference of the Aslib Transport and Planning Group** (Mr C. C. Parker, The Library, University of Southampton, Southampton SO9 5NH, UK)

April 22-25, **Joint American Physical Society and Optical Society of America Meeting** (The American Institute of Physics, 335 East 45 Street, New York 10017)

April 22-26, **Biochemische Analytik 74** combined with **1st European Congress of Clinical Chemistry** (Secretary General, Dr Rosmarie Vogel, D-8000 Munchen 2, Nussbaumstrasse 20, West Germany)

April 22-26, **Conference on Anomalous Scattering** (S. C. Abrahams, (International Union of Crystallography), Bell Laboratories, Murray Hill, New Jersey 07974)

April 22-26, **The Engineer in Society** (EUROCON '74, c/o Klvl, 23 Prinsessegracht, The Hague, The Netherlands)

April 22-26, **European Conference on Electrotechnics** (Mr G. Gaikhorst, c/o FME, Nassaulaan 13, The Hague, The Netherlands)

April 22-May 3, **Exploitation of Seismograph Networks** (International Advanced Study Institute, Sandefjord, Norway)

April 24, **Easter Lecture for Young People: Instant Energy-Heat Release with a Bang** (L. F. Linnett, Lecture Secretary, The Institute of Fuel, St Bernards House, St Bernards Road, Tonbridge, Kent TN10 3NL)

April 24-25, **Conference on Rheology of Clays and Cement Pastes** (Dr G. H. Tattersall, Department of Building Science, University of Sheffield, Arts Tower, Sheffield S10 2TN)

April 28-May 2, **76th Annual Meeting and Exposition of the American Ceramic Society** (Dr Peter Hawkins, Program Chairman, California Portland Cement Company, Box 947, Colton California 92324)

April 30-May 2, **3rd International Cannabis Conference** (The Conference Secretary, Institute for the Study of Drug Dependence, Chandos House, 2, Queen Anne Street, London W1M 0BR)

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Council for Academic Freedom and Democracy. Annual Report 1972-1973. Pp. 16. (London: Council for Academic Freedom and Democracy, 1973.) [1511]
 Meteorological Office. Geophysical Memoirs No. 119: A Climatology of the Stratosphere Over North-West Europe. By R. A. Hamilton, B. D. Mason and G. C. Bridge. (Met.O.864a.) Pp. 37. (London: HMSO, 1973.) £2.10 net. [1611]
 Arthritis: a Vitamin Deficiency Disease. By Dr E. C. Barton-Wright. Pp. 32. (London: United Trade Press Ltd, 1973.) £1.25. [412]
 The Pittdown Man Hoax. (Palaeontology Leaflet No. 2.) Pp. 7. (London: British Museum (Natural History), 1973.) 7p. [412]
 Patterns of Growth. By Jerome S. Bruner. (Inaugural Lecture delivered before the University of Oxford on 25 May 1973.) Pp. 22. (Oxford: Clarendon Press, 1974.) 50p net. [612]
 The Medical Research Council of Ireland. Annual Report for the year ended December 31, 1972. Pp. 106. (Dublin: Medical Research Council of Ireland, 1973.) 25p. [612]
 British Antarctic Survey. Scientific Reports, No. 67: The Geology of Parts of the Bowman and Wilkins Coasts, Antarctic Peninsula. By A. G. Fraser and P. H. Grimley. Pp. 59+8 plates. (London: British Antarctic Survey, 1972.) £2.80 net. [712]

Other Countries

An Annotated Bibliography, 1797-1969. By Warren Addicott. Pp. iii+201. \$1.50. Bulletin 1374: Placer Deposits of Alaska. By Edward H. Cobb. Pp. vi+231+plate 1. \$3.10. Water-Supply Paper 2019-B: Generalization of Stream-Temperature Data in Washington. By M. R. Collings. Pp. iv+45. 30 cents. Water-Supply Paper 2092: Quality of Surface Waters of the United States, 1968. Part 2: South Atlantic Slope and Eastern Gulf of Mexico Basins. Pp. x+373. \$2.35. Professional Paper 526-C: Cretaceous and Early Tertiary Depositional and Tectonic History of the Livingstone Area, Southwestern Montana. By Albert E. Roberts. Pp. iv+120+plates 1-3. Professional Paper 306-E: Geology and Paleontology of Canal Zone and Adjoining Parts of Panama. Description of Tertiary Mollusks (Additions to Gastropods, Scaphopods, Pelecypods—Nuculidae to Malleidae). By W. P. Woodring. Pp. iii+453-539+plates 67-82. \$1.75. (Washington, DC: Government Printing Office, 1973.) [3010]
 Animal Models of Human Disease: a Handbook. Pp. 98. (Reprints.) (Washington, DC: The Registry of Comparative Pathology, Armed Forces Institute of Pathology, 1973.) [3110]
 Smithsonian Contributions to Zoology. No. 120: A Systematic Monograph of New World Ethmid Moths (Lepidoptera: Gelechioidea). By Jerry A. Powell. Pp. iv+302. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) \$3.85. [3110]
 Nederlandse Vereniging voor Weer- en Sterrenkunde. Observations of Variable Stars, January-June 1973. (Report No. 24.) Pp. 7. (Groningen, Netherlands: Kapteyn Astronomical Laboratory, 1973.) [3110]
 Smithsonian Contributions to the Earth Sciences, No. 10: Mineralogy, Mineral-Chemistry, and Composition of the Murchison (C2) Meteorite. By Louis H. Fuchs, Edward Olsen and Kenneth J. Jensen. Pp. iii+39. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office, 1973.) 75 cents. [611]
 Ecology and Resource Development in Southeast Asia: a Report to the Ford Foundation. By Gordon Conway and Jeff Romm. Pp. 82. (New York: Ford Foundation, 320 East 43 Street, 1973.) [711]
 Smithsonian Contributions to Zoology. No. 150: A Review of the Genus *Cancellus* (Crustacea: Diogenidae) with the Description of a New Species from the Caribbean Sea. By Barbara Shuler Mayo. Pp. iii+63. \$1.50. No. 151: Revision of Corophiidae and Related Families (Amphipoda). By J. Laurens Barnard. Pp. iv+27. 55 cents. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) [711]
 United States Department of the Interior: Geological Survey. Bulletin 1351: Geology and Description of the Thorium-Bearing Veins, Lemhi Oass Quadrangle, Idaho and Montana. By Mortimer H. Staatz. Pp. iv+94+plates 1-4. Professional Paper 716-E: Distribution, Thickness and Lithology of Paleocene Rocks in Pakistan. By Charles R. Meissner, Jr., and Habib-ur Rahman. Pp. v+6+plates 1-4. \$1.25. (Washington, DC: Government Printing Office, 1972 and 1973.) [711]
 The Coconut Industry Board, Jamaica, West Indies. 12th Report of the Research Department, July 1971-June 1972. Pp. 65. (Kingston: Coconut Industry Board, 1973.) \$52; 90p: US\$2.20. [811]
 Bulletin of the American Museum of Natural History. Vol. 152, Article 2: Revision of Ground Beetles of American Genus *Cychrus* and Four Subgenera of Genus *Scaphinotus* (Coleoptera, Carabidae). By Tatiana Gidaspow. Pp. 51-102. (New York: American Museum of Natural History, 1973.) \$23.00. [1311]